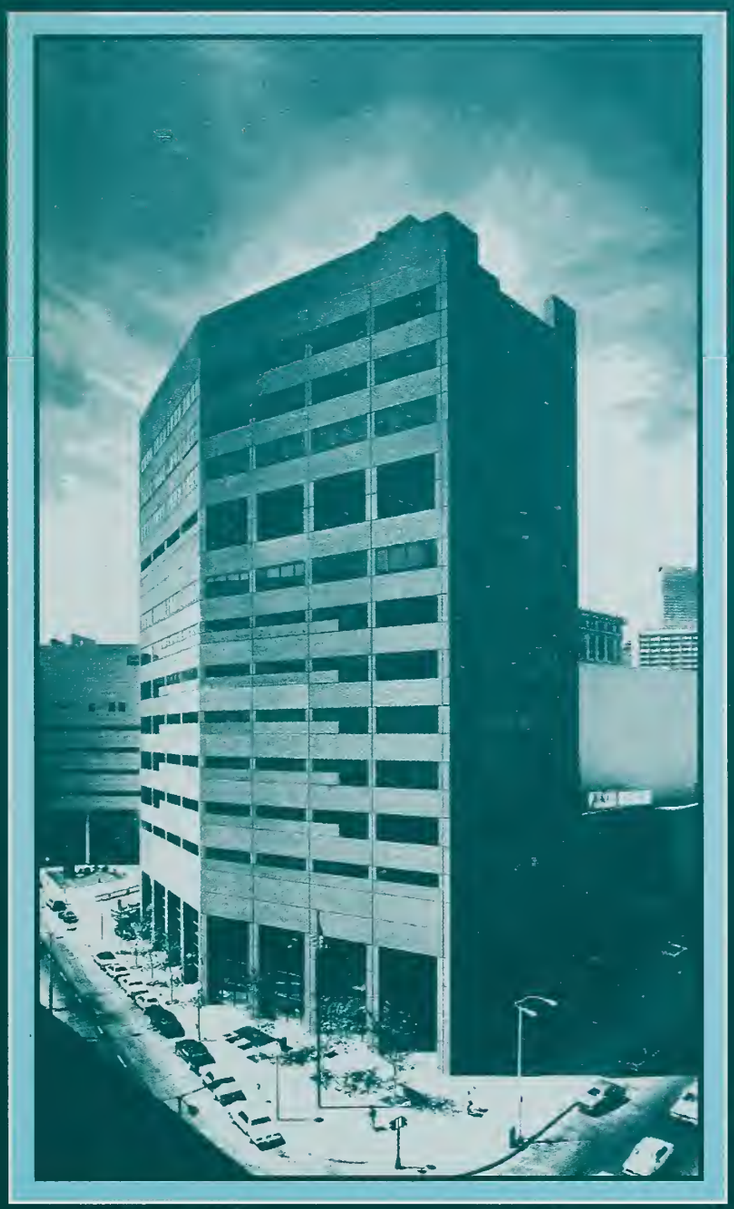
JEAN MAYER
UNITED STATES DEPARTMENT OF AGRICULTURE
HUMAN NUTRITION RESEARCH CENTER ON AGING AT TUFTS UNIVERSITY



RESEARCH PROGRAM DESCRIPTION

711 Washington Street
Boston, Massachusetts 02111

Jean Mayer
United States Department of Agriculture

Human Nutrition Research Center on Aging
at Tufts University

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INTRODUCTION

The Jean Mayer United States Department of Agriculture (USDA) Human Nutrition Research Center on Aging (HNRC) was established by Congress through the Food and Agricultural Act of 1977 as one of five mission-oriented research centers designed to study the effect of human nutrition on health. Since 1982, the HNRC has been operated by Tufts University under the authority of the USDA as a government-owned, contractor-operated facility.

The creation of the HNRC was a major response of the federal government to the growing awareness of the need for improved nutrition recommendations for the American public throughout the life cycle. The overall mission of the HNRC is to explore the relationship between nutrition and good health and to determine the nutritional and dietary requirements of the maturing and elderly population. The interaction between nutrition and the onset and progression of aging and associated degenerative conditions is of special concern. HNRC scientists conduct cell and molecular biology, animal model, and human metabolic and field studies to further their understanding of the processes of nutrient utilization and metabolism to determine ways by which diet, in combination with genetic and environmental factors may promote health and vigor over the lifespan.

HNRC investigators address three general questions of central importance to their mission:

- *What are the nutrient requirements necessary to obtain optimal function and well being for a maturing population?*
- *How does nutrition influence the progressive loss of tissue function associated with aging?*
- *What is the role of nutrition in the genesis of the major chronic, degenerative conditions associated with the aging process?*

The proximity of the HNRC to the Tufts Schools of Medicine, Dental Medicine and Sackler School of Graduate Biomedical Sciences on the Tufts Health Sciences campus in downtown Boston, offers the opportunity for HNRC scientists to pursue collaborations with other university investigators. Most science track graduate students enrolled in the Tufts University School of Nutrition Science and Policy obtain their training under the supervision and guidance of HNRC researchers.

This booklet lists the HNRC investigators and provides highlights of their scientific expertise and current research interests. Descriptions of the sixteen laboratory programs which are administratively described by the USDA through Current Research Information System (CRIS) units are provided. The capabilities and services of the Center's eight specialized core service units are also described. This information is intended to acquaint the reader with the scope of research efforts and some recent achievements but does not comprehensively embrace aspects of HNRC research efforts.

For additional information please refer to the HNRC Web site: *http://www.hnrc.tufts.edu*

Table of Contents

Bess Dawson-Hughes, M.D. *(Tufts University School of Medicine)*
Chief, Calcium and Bone Metabolism Laboratory; Scientist I. Physician trained in Internal Medicine and Endocrinology. Laboratory expertise in clinical study design and data interpretation, bone densitometry, clinical trials, calcium absorption, intake assessment. Areas of research interest include age-related change in dietary and hormonal regulation of calcium and bone metabolism and their relationship to the pattern of bone loss in men and women.

William H. Dietz, M.D., *(University of Pennsylvania)* Ph.D. *(Massachusetts Institute of Technology)*
Scientist I, Epidemiology Program. Board certified pediatrician with research interests in energy metabolism and body composition relationships and between growth patterns and later morbidity/mortality.

Gregory G. Dolnikowski, Ph. D. *(Michigan State University)*
Chief, Mass Spectrometry Laboratory; Scientist II. Analytical chemist trained in Mass Spectrometry. Laboratory expertise in gas chromatography/mass spectrometry, tandem mass spectrometry, instrument development, gas-phase ion chemistry, isotope ratio analysis, and trace organic analysis. Research interests include energy expenditure determination by the doubly labeled water method, trace analysis of nutrients by GC/MS and LC/MS, and analytical instrument development.

Johanna Dwyer, D.Sc., *(Harvard University)* **R.D.**
Senior Scientist, Epidemiology Program. Nutritionist with expertise in diet and chronic degenerative conditions, and dietary methodology. Current research efforts in the examination of the associations of diet with subsequent health, especially renal function and body composition; and the validity of dietary intake measures and recall accuracy for autobiographical data, especially diet and body weight.

Maria A. Fiatarone, M.D. *(University of California - San Diego)*
Chief, Nutrition and Exercise Physiology Laboratory; Scientist I. Board certified in Internal Medicine and trained in geriatric medicine. Research focuses on the effects of exercise and nutrient status on the functional capacity of institutionalized elderly.

Roger A. Fielding, Ph.D. *(Tufts University)*
Scientist III, Nutrition and Exercise Physiology Laboratory. Physiologist with research interests in the effects of concentric and eccentric muscle actions on changes in protein turnover.

James C. Fleet, Ph.D. *(Cornell University).*
Scientist II. Mineral Bioavailability Laboratory. Nutritional biochemist with expertise in cellular and molecular biology. Studies focus on using various cell culture and animal models to investigate the effects of aging and hormonal status on bone and mineral metabolism.

Xin Gong, M.D. *(Nanjing Medical College, China)*
Research Associate, Laboratory for Nutrition and Vision Research. Studies relationships between oxidative stress, nutrient antioxidation function, and protein metabolism during aging.

Andrew S. Greenberg, M.D. *(New York University)*
Scientist II, Energy Metabolism Laboratory. Physician/endocrinologist with research interests in the nutritional, cellular, hormonal, and genetic aspects of energy regulation, obesity, and adipose tissue and the interaction of body composition and aging.

Ohkee Han, Ph.D. *(University of North Carolina at Greensboro)*
Research Associate, Mineral Bioavailablity Laboratory. Conducts animal and Caco-2 cells studies on the mechanism of intestinal iron transport and the role of abnormal regulation of iron absorption in genetic iron overload.

Garry J. Handelman, Ph.D. *(Tufts University)*
Scientist II, Antioxidants Research Laboratory. Nutritional biochemist with research interests in the biochemical basis for the role of antioxidant nutrients and their dietary requirements in health promotion and disease prevention during the aging process.

Wilburta J. Hartman, Ph.D. *(University of California - Davis)*
USDA Nutritionist, Amino Acid Metabolism Laboratory. Nutritionist with expertise in amino acid requirements, metabolic effects of amino acid deficient diets and gastrointestinal function and food intake assessment of human populations.

Virginia Hughes, M.S. *(Boston University)*
Research Associate, Nutrition and Exercise Physiology Laboratory. Physiologist with research interests on the effects of high carbohydrate/high fiber diet on glucose metabolism.

Paul F. Jacques, D.Sc. *(Harvard University)*
Associate Chief, Epidemiology Program; Scientist I. Epidemiologist with expertise in study design, implementation, and data analysis for epidemiologic, clinical and laboratory studies. Current research examines the role of antioxidant nutrients in the prevention or delay of onset of age-related macular degeneration and opacification of the eye lens; the influence of vitamin C intake on cardiovascular disease risk factors; and the role of B vitamins and methionine metabolism in modifying vascular disease risk.

Elizabeth J. Johnson, Ph.D. *(University of Wisconsin)*
Scientist II, Gastrointestinal Nutrition Laboratory. Nutritional biochemist with expertise in retinoid and carotenoid metabolism especially in fat and intestine.

James A. Joseph, Ph.D. *(University of South Carolina)*
Chief, Neuroscience Laboratory. Neuropharmacologist with expertise in characterizing the mechanism involved in neuronal changes in senescence which are expressed as decrements in motor behavioral and cognitive behaviors.

Joseph J. Kehayias, Ph.D. *(Indiana University)*
Chief, Body Composition Laboratory; Scientist I. Nuclear physicist with expertise in application of nuclear instrumentation to *in vivo* body composition analysis. Current research involves development of new body composition models, *in vivo* measurements of body fat and studies of body composition changes with aging, diet, and physical activity.

Patricia Khu, M.D. *(University of the Philippines)*
Consultant, Laboratory for Nutrition and Vision Research. Ophthalmologist with interests in the relationships between nutrition and development of age-related eye diseases.

Elizabeth A. Krall, Ph.D. *(University of Pittsburgh)*
Scientist II, Calcium and Bone Metabolism Laboratory. Trained in biology and epidemiology. Research expertise in conducting clinical trials of environmental and hereditary factors which influence patterns of bone development and loss; data analysis; and interpretation.

Norman I. Krinsky, Ph.D. *(University of Southern California)*
Senior Scientist, Gastrointestinal Nutrition Laboratory. Biochemist with research interests in carotenoid function and metabolism; role of carotenoids in human macula. Expertise in carotenoid, tocopherol and retinoid analyses.

Stefania Lamon-Fava, M.D., *(University of Padova School of Medicine, Italy)* **Ph.D.** *(University of Modena, Italy)*
Scientist II, Lipid Metabolism Laboratory. Physician with research interest in the epidemiological examin-

ation of studies on nutritional, hormonal, and genetic regulation of plasma lipoproteins. Examines the nutritional and hormonal control of apolipoprotein A-I gene expression.

Alice H. Lichtenstein, D.Sc. *(Harvard University)*
Scientist I, Lipid Metabolism Laboratory. Nutritional biochemist with interest in the nutritional regulation of plasma lipoprotein metabolism *in vivo*. Research involves the effects of dietary fatty acids and cholesterol on lipoprotein composition and apolipoprotein metabolism in human and non-human primates.

Ruth Lipman, Ph.D. *(Worcester Polytechnic Institute)*
Scientist II, Antioxidants Research Laboratory. Biochemist interested in biomarker of aging studies in animal models. Expertise in the growth of cells *in vitro* (including clonal culture), chromosome banding, micromanipulation of individual cellular components, signals for cellular replication, and ion transport.

Keith R. Martin, Ph.D. *(University of North Carolina at Greensboro)*
Research Associate, Vascular Biology Program. Nutritional biochemist with expertise in nutrient modulation of oxidative stress and antioxidant balance in human cell lines, with particular interest in the role of carotenoids as potential cellular antioxidants.

Joel B. Mason, M.D. *(University of Chicago)*
Scientist I, Vitamin Metabolism Laboratory. Physician trained in nutrition and gastroenterology. Current research focus on intestinal absorption with emphasis on mechanism and effects of drugs.

Mohsen Meydani, D.V.M., *(Tehran University, Iran),* **Ph.D.** *(Iowa State University)*
Senior Scientist, Vascular Biology Program. Nutritional biochemist with expertise in antioxidants and oxidative stress with special emphasis on dietary fat and antioxidant modulation of oxidative stress in aging and vascular biology. Current research focuses on antioxidant effects on immune/endothelial cell interactions.

Simin Nikbin Meydani, D.V.M., *(Tehran University, Iran)* **Ph.D.** *(Iowa State University)*
Chief, Nutritional Immunology Laboratory; Senior Scientist. Nutritional immunologist with research interest in the role of nutrients in the control of the immune response under physiological and pathological conditions with special emphasis on dietary fats and antioxidants and a focus on dietary modification of eicosanoid production and biologic function.

Aviva Must, Ph.D. *(Tufts University)*
Scientist II, Epidemiology Program. Epidemiologist with research interests in the assessment of the influence of early body composition on mortality and morbidity among older persons and the delineation of the role of reproductive factors on the incidence and maintenance of obesity in women.

Miriam Nelson, Ph.D. *(Tufts University)*
Associate Chief, Nutrition and Exercise Physiology Laboratory; Scientist II. Nutritionist with research interests on the effects of exercise and diet on bone health and body composition in the elderly.

Martin Obin, Ph.D. *(University of Florida)*
Scientist II, Laboratory for Nutrition and Vision Research. Cell physiologist and biochemist with research interests in the relationship between light exposure, nutriture, and protein turnover in the retina or cultured retinal cells; and the mechanisms of neuronal cell differentiation and regeneration.

Jose M. Ordovas, Ph.D. *(University of Zaragoza, Spain)*
Scientist I, Lipid Metabolism Laboratory. Biochemist and molecular biologist with research interest in the metabolism and genetics of lipoproteins and apolipoproteins, particularly the interrelationship of nutritional and genetic factors associated with lipoprotein abnormalities and premature atherosclerosis.

Helen Palmer, Ph.D. *(Michigan State University)*
Scientist III, Genetics Laboratory. Nutritional biochemist with experience in cell biology and molecular biology. Investigates age-related changes in signal transduction and gene expression in the mammalian liver. Specific focus is on alterations in membrane lipid/vitamin E composition with aging and how these changes affect MAP kinase cascades and AP-1 gene activation.

K. Eric Paulson, Ph.D. *(University of California - Davis)*
Chief, Genetics Laboratory/Scientist II. Molecular biologist with research interests in the metabolism and genetic regulation of lipogenesis and liver function.

Ronald L. Prior, Ph.D. *(Cornell University)*
USDA Scientific Program Officer/Chief, Amino Acid Metabolism Laboratory. Nutritional biochemist with postgraduate certification in comparative gastroenterology. Areas of research interest include gastrointestinal amino acid absorption and metabolism.

Judy Ribaya-Mercado, D.Sc. *(Harvard University)*
Scientist II, Gastrointestinal Nutrition Laboratory. Nutritionist with interest in the effects of vitamin B-6 on carbohydrate and lipid metabolism. Currently researching the effects of age, atrophic gastritis, and intestinal flora on human water soluble vitamin requirements.

Karen Riggs, Ph.D. *(University of Texas - Austin)*
Scientist III, Epidemiology Program. Psychologist with research interests in the effect of nutrition on cognitive aging and the relationship of personality and cognitive abilities to the accuracy of dietary self-report.

Susan B. Roberts, Ph.D. *(University of Cambridge, England)*
Chief, Energy Metabolism Laboratory; Scientist I. Physiologist with expertise in studies of energy metabolism and obesity. Areas of research interest include energetic efficiency, energy expenditure, and energy intake in humans, and the stable isotopes kinetic tech-niques to study metabolism.

Ronenn Roubenoff, M.D., *(Northwestern University Medical School)* **M.H.S.** *(Johns Hopkins University)*
Scientist I, Sarcopenia Research Program. Rheumatologist/epidemiologist with research interests in interactions of body composition and immune function, with emphasis on chronic inflammation and normal aging.

Irwin H. Rosenberg, M.D. *(Harvard Medical School)*
HNRC Director; Program Director, Bioavailability Laboratories; Senior Scientist. Gastroenterologist with research interests in intestinal absorption and bioavailability of vitamins, and the relationship among homocysteine, vascular disease and vitamin nutrition.

David Rush, M.D. *(Harvard Medical School)*
Chief, Epidemiology Program; Senior Scientist. Epidemiologist/pediatrician with research interests in the effects of nutritional status and diet on health, with early life experience on later nutritional and health status; nutritional screening in the elderly; and the impact of socioeconomic forces on nutritional status. Retains a strong interest in program evaluation as well as classical epidemiology.

Robert M. Russell, M.D. *(Columbia University)*
Associate Director; Chief, Gastrointestinal Nutrition Laboratory; Senior Scientist. Clinical nutritionist certified in Gastroenterology and Internal Medicine. Current research interests include biochemical and functional testing in protein, folic acid, and vitamin A malnutrition, carotenoid metabolism and alcoholism in animal models and humans.

James Sadowski, Ph.D. *(University of Wisconsin)*
Chief, Vitamin K Laboratory; Scientist I. Nutritional biochemist with experience in fat-soluble vitamin and essential fatty acid metabolism and function, protein purification and characterization, and cell culture. Research interests focus on the metabolism and function of vitamin K with age-related changes in requirements and metabolism and proteins involved in blood clotting and bone formation.

Robert N. Salomon, M.D. *(University of Massachusetts)*
Scientist II, Vitamin Metabolism Laboratory. Physician board certified in Anatomic and Clinical Pathology with expertise in immunopathology and nucleic acid hybridization techniques. Current areas of research interest include the molecular and cellular biology of the aging cardiovascular system and the role of diet in the development of age-related cardiovascular diseases.

Edward Saltzman, M.D. *(University of Rochester)*
Scientist III, Energy Metabolism Laboratory. Physician with research interests in the role of inheritance in energy metabolism and its genetic determinants.

John Saltzman, M.D. *(University of Massachusetts - Worcester)*
Visiting Scientist, Gastrointestinal Nutrition Laboratory. Physician with research interests in the areas of atrophic gastritis and intestinal bacteria overgrowth. Studies adherent strains of lactobacilli which can colonize the upper gastrointestinal tract of elderly adults.

Ernst J. Schaefer, M.D. *(Mt. Sinai School of Medicine)*
Chief, Lipid Metabolism Laboratory; Senior Scientist. Physician trained and certified in Internal Medicine, Endocrinology and Metabolism. Laboratory expertise in lipoprotein biochemistry and metabolism. Areas of research interest include age-related changes in dietary, hormonal, and genetic regulation of plasma lipoproteins and the relationship of lipoprotein levels to the risk of premature atherosclerosis and longevity.

Jacob Selhub, Ph.D. *(Case Western Reserve University)*
Chief, Vitamin Metabolism Laboratory; Senior Scientist. Biochemist with expertise in vitamin absorption and metabolism and vitamin binding proteins. Research interest in folate nutriture.

Leo Seman, M.D., Ph.D. *(Dalhouise University, Nova Scotia)*
Research Associate, Lipid Metabolism Laboratory. Physician with research interest in the nutritional and genetic regulation of lipoprotein (a).

Fu Shang, Ph.D. *(Beijing Medical University)*
Scientist II, Laboratory for Nutrition and Vision Research. Research interests include the *in vivo* determination of the efficacy of nutrients to delay cataract in laboratory animals, and protease function and expression as affected by oxidative stress and nutrients.

Gwang-Wen Tang, Ph.D. *(Rutgers University)*
Scientist II, Gastrointestinal Nutrition Laboratory. Organic chemist with expertise in hydrogen transfer oxidation and reduction reactions. Extensive expertise with HPLC, GC, MS, NMR, AA and X-ray diffraction analysis.

Allen Taylor, Ph.D. *(Rutgers University)*
Chief, Nutrition and Vision Research Laboratory; Senior Scientist. Biochemist with interests in protein degradation and protein aging in the eye. Experience in epidemiological/clinical studies and nutritional/biochemical program design. Laboratory expertise with NMR, IR, UV-VIS spectroscopy, X-ray diffraction, electron-microscopy of macromolecular assemblies, protein purification, eye lens histology, immunofluorescence, immunological techniques. Studies involve molecular cell and organ culture, and *in vivo* approaches.

Katherine Tucker, Ph.D. *(Cornell University)*
Scientist I, Epidemiology Program. Nutritional epidemiologist with expertise in study design and data analysis for population-based research. Current research areas include dietary methodology, diet and disease relationships, social and behavioral factors related to diet and nutritional status of the elderly; and nutrition and health among Hispanic elderly.

Xian-Dong Wang, M.D., *(Beijing Medical University, China)* **Ph.D.***(Tufts University)*
Scientist II, Gastrointestinal Nutrition Laboratory. Physician and biochemist with research interest in absorption and metabolism of retinoids and carotenoids in both human and animal models, and a focus on the isomers of ß-carotene metabolism and interaction with vitamin E.

Francine Welty, M.D., Ph.D. *(Case Western Reserve University)*
Scientist II, Lipid Metabolism Laboratory. Physician with research interest in examination of the factors regulating the genetic control of plasma low density lipoprotein response, especially apoB gene mutations to dietary modification.

Richard J. Wood, Ph.D. *(University of Connecticut).*
Chief, Mineral Bioavailability Laboratory; Scientist I. Nutritional physiologist with expertise in age-related changes in intestinal mineral metabolism. Areas of research interest include the pathogenesis of osteoporosis, age-related changes in intestinal mineral absorption and requirements in humans, and the effects of diet and hormonal status on the expression of proteins which modulate cellular mineral metabolism.

Kyung-Jin Yeum, Ph. D. *(Yonsei University)*
Research Associate, Gastrointestinal Nutrition Laboratory. Scientist with research interests in the effects of lipoxagenases on carotenoid metabolism. Also studies carotenoid levels in lens tissue.

Dayong Wu, M.D. *(North Bethune University, China)*
Scientist III, Nutritional Immunology Laboratory. Physiologist with research interest in the physiological effects of polyunsaturated fatty acids with expertise in the assay of eicosanoid metabolites in biological samples.

CORE SERVICE UNITS

Dietary Assessment Unit

The Dietary Assessment Unit provides dietary assessment services for HNRC nutrition studies. These services include collecting, checking, coding, and quality control for various dietary methodologies including food diary records, 24-hour recalls and food frequency questionnaires. The unit provides training for investigators who choose to collect and process their own dietary data and participates in research and development related to dietary assessment methodology. Food and nutrient information is derived from the Minnesota Nutrition Data System software developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN. The unit is equipped with an optical scanner (Op Scan 5, National Computer Systems, Minneapolis, MN) for processing a version of the National Cancer Institute's Food Frequency Questionnaire.

DIETARY ASSESSMENT STAFF

Katherine Tucker, Ph.D
Scientist I
Director, Dietary Assessment Unit

Develops and implements new dietary assessment services. Establishes scientific priorities for nutrient database and departmental services.

Janice Maras, B.A., Dietary Data Manager
Peter Bakun, B.S., Nutritional Data Coordinator
Peter Zhu, M.S., Programmer/Analyst

SELECTED RECENT PUBLICATIONS

Booth SL, Sokoll JL, O'Brien ME, Tucker K, Dawson-Hughes B, Sadowski JA. Assessment of dietary phylloquinone intake and vitamin K status in postmenopausal women. Eur J Clin Nutr 1995;49:832-41.

Tucker KL, Dallal GE, Rush D. Dietary patterns of elderly Boston-area residents defined by cluster analysis. J Am Diet Assoc 1992;92:1487-91.

Tucker K, Rush D. Food choices of the elderly. In: Hartz SC, Russell RM, Rosenberg IH, eds. Nutrition in the elderly: the Boston Nutritional Status Survey. London: Smith-Gordon and Company Limited, 1992:45-54.

Division of Biostatistics

The Division of Biostatistics consults and assists in all phases of study design, implementation, and analysis. Researchers are encouraged to confer with the Division early in the planning of a study to review project goals in relation to available resources and accepted statistical practice. The Division assists investigators in the choice of response variables and covariates that can be subjected to proper statistical analysis and constructs plans for randomizing subjects to treatment and may serve as the custodian of the randomization scheme for double-blind protocols. In addition, training and assistance is provided to enable investigators to perform their own routine data analyses. New statistical techniques and statistical software are developed in support of research activities.

BIOSTATISTICS STAFF

Gerard E. Dallal, Ph.D.
Scientist I
Associate Professor, Nutrition

Offers consultation and workshops on statistical topics and software packages. Serves as a preliminary manuscript reviewer to help researchers address questions that may arise during the refereeing process. Assists in resolving issues that occur during the peer review of submitted articles.

SELECTED RECENT PUBLICATIONS

Pedrosa MC, Golner BB, Goldin BR, Barakat S, Dallal GE, Russell RM. Survival of yogurt-containing organisms and *Lactobacillus gasseri* (ADH) and their effect on bacterial enzyme activity in the gastrointestinal tract of healthy and hypochlorhydric elderly subjects. Am J Clin Nutr 1995;61:353-8.

Morganti CM, Nelson ME, Fiatarone MA, Dallal GE, et al. Strength improvements with 1 yr of progressive resistance training in older women. Med Sci Sports Exerc 1995;27:906-12.

Dawson-Hughes B, Harris SS, Krall EA, Dallal GE, et al. Rates of bone loss in postmenopausal women randomly assigned to one of two dosages of vitamin D. Am J Clin Nutr 1995;61:1140-5.

Roubenoff R, Dallal GE, Wilson PWF. Predicting body fatness: the Body Mass Index vs estimation by bioelectric impedance. Am J Public Health 1995;85:726-38.

Roberts SB, Dietz W, Sharp T, Dallal GE, Hill JO. Multiple laboratory comparisons of the doubly labeled water technique. Obes Res 1995;3(Suppl 1):3-14.

Taylor A, Lipman RD, Jahngen-Hodge J, et al. Dietary calorie restriction in the Emory mouse: Effects on lifespan, eye lens cataract prevalence and progression, levels of ascorbate, glutathione, glucose, and glycohemoglobin, tail collagen breaktime, DNA and RNA oxidation, skin integrity, fecundity, and cancer. Mech Ageing Dev 1995;79:33-57.

Hughes VA, Frontera WR, Dallal GE, et al. Muscle strength and body composition: associations with bone density in older subjects. Med Sci Sports Exerc 1995;27:967-74.

Campbell WW, Crim MC, Dallal GE, et al. Increased protein requirements in elderly people: new data and retrospective reassessments. Am J Clin Nutr 1994; 60:501-9.

Lyu L-C, Shieh M-J, Posner BM, et al. Relationship between dietary intake, lipoproteins, and apolipoproteins

in Taipei and Framingham. Am J Clin Nutr 1994;60:765-74.

Roberts SB, Fuss P, Heyman MB, et al. Control of food intake in older men. JAMA 1994;272:1601-6.

Saltzman JR, Kemp JA, Golner BB, Pedrosa MC, Dallal GE. Effect of hypochlorhydria due to omeprazole treatment or atrophic gastritis on protein-bound vitamin B_{12} absorption. J Am Coll Nutr 1994;6:584-91.

Lyu LC, Shieh MJ, Bailey S, Dallal GE, et al. Relationship of body fat distribution with cardiovascular risk factors in healthy Chinese. Ann Epidemiol 1994;4:434-44.

Dawson-Hughes B, Harris S, Kramich C, Dallal G, Rasmussen HM. Calcium retention and hormone levels in black and white women on high- and low-calcium diets. J Bone Miner Res 1993;8:779-87.

Hegsted DM, Ausman LM, Johnson JA, Dallal GE. Diet, fat, and serum lipids: and evaluation of the experimental evidence. Am J Clin Nutr 1993;57:875-83.

Dallal GE, Rousseeuw PJ. LMSMVE: A program for least median of squares regression and robust distances. Comp Biomed Res 1992;25:384-91.

Must A, Jacques PJ, Dallal GE, Bajema CJ, Dietz WH. Long-term morbidity and mortality of adolescent obesity: a follow-up of the Third Harvard Growth Study of 1922 to 1935. New Engl J Med 1992;327:1350-5.

Tucker KL, Dallal GE, Rush D. Dietary patterns of elderly Boston-area residents defined by cluster analysis. J Am Diet Assoc 1992;92:1487-91.

Harris S, Dallal GE, Dawson-Hughes B. Influence of body weight on rates of change in bone density of the spine, hip and radius in post menopausal women. Calcif Tissue Int 1992;50:19-23.

Division of Comparative Biology and Medicine

The Division of Comparative Biology and Medicine (CB&M) is comprised of a 25,000 square foot animal research laboratory which encompasses 28 barrier-type animal rooms on two secured levels. The core facility is designed for an all inclusive clean-dirty corridor system with anteroom extensions in approximately half of the rooms. A dedicated elevator for animal transport provides an extension of the clean-dirty corridor system. Strict guidelines are established for environmental control including temperature and humidity, air exchange with appropriate positive or negative pressure, and duration and intensity of illumination. The air exchange system is balanced to provide 15 complete changes/hour/room of 100 percent fresh, non-recirculated air at a mass displacement, low velocity air flow. Environmental conditions are monitored on a 24-hour basis. All animal rooms are supplied with continuous flowing, minimal bioburden, deionized water which can be used selectively from a closed automatic watering loop. Ten animal rooms are designed to permit experimental work with bio-hazardous agents requiring Class Three Containment per standards set by the Centers for Disease Control.

The CB&M possesses fully equipped surgery and necropsy suites and a specialized animal diet kitchen. The unit offers a wide range of veterinary support services including diet preparation, veterinary nurse and research assistance, surgery, necropsy, and clinical pathology. Comprehensive veterinary surveillance diagnostic testing is provided by the Charles River Breeding Laboratories. The facility complies fully with all applicable provisions of the Animal Welfare Act and other related federal, state and local statutes, regulations and general provisions for animal welfare including standards identified by the NIH Guide of the Care and Use of Laboratory Animals. The HNRC has been fully accredited by the American Association for Accreditation of Laboratory Animal Care since its inception in 1983. All animal studies are reviewed and approved by a duly constituted Animal Care and Use Committee per guidelines of the National Institutes of Health Office for the Protection from Research Risks.

COMPARATIVE BIOLOGY AND MEDICINE STAFF

Donald Smith, M.S., R.L.A.T. Manager	Provides administrative and scientific direction to ensure compliance with research protocols and government regulations. Liaison between veterinary, research and administrative staff.
Roderick Bronson, D.V.M., D.A.C.V.P. Veterinarian, Scientist I Associate Professor, Pathology	Provides veterinary diagnostic, therapeutic and pathology services. Expertise in veterinary and research pathology of the aging processes, genetic diseases and neuropathology.

Maureen Kelliher, B.A., Laboratory Animal Technician
Jonathan Morrison, M.S., Laboratory Animal Technician
Anthony Sealy, Laboratory Animal Technician
Janice Williams, Histologist
Shannon Hacker, Staff Assistant

SELECTED RECENT PUBLICATIONS

Taylor A, Lipman RD, Jahngen-Hodge J, Palmer V, et al. Dietary calorie restriction in the Emory mouse: Effects on lifespan, eye lens cataract prevalence and progression, levels of ascorbate, glutathione, glucose, and glycohemoglobin, tail collagen breaktime, DNA and RNA oxidation, skin integrity, fecundity, and cancer. Mech Ageing Dev 1995;79:33-57.

Smith DE, Rizzone G, Blumberg JB. Use of epinephrine auto-injectors for acute anaphylactic reactions in an animal care facility. Contemporary Topics 1995;34:99-102.

Taylor A, Jahngen-Hodge J, Smith DE, et al. Dietary restriction delays cataract and reduces ascorbate levels in Emory mice. Exp Eye Res 1995;61:55-62.

Mune M, Meydani M, Jahngen-Hodge J, Martin A, Smith DE, et al. Effect of caloric restriction on liver and kidney glutathione in aging Emory mice. AGE 1995;18:43-9.

Lipman RD, Smith DE, Bronson RT, Blumberg JB. Is late-life caloric restriction beneficial? Aging Clin Exp Res 1995;7:136-9.

Robins SJ, Fasulo JM, Patton GM, Schaefer EJ, Smith DE, et al. Gender differences in the development of hyperlipidemia and atherosclerosis in hybrid hamsters. Metabolism 1995;44:1326-31.

Kim Y-I, Christman JK, Fleet JC, et al. Moderate folate deficiency does not cause either global hypomethylation of hepatic and colonic or c-*myc* specific hypomethylation of colonic DNA in rats. Am J Clin Nutr 1995; 61:1083-90.

Smith D, Palmer V, et al. Induction of cataracts by x-ray exposure of guinea pig eyes. Lab Anim 1993;22(3):34-9.

Taylor A, Smith DE, Palmer VJ, et al. Relationships between acetone, cataracts and ascorbate in hairless guinea pigs. Ophthalmol Res 1993;25:30-5.

Smith D, Morrow F, Patnaude D. A program for safe carcinogen use in research rodents. Canadian Association for Laboratory Animal Science/ACTAL 1993;27(1):5-9

Smith D, Bronson R. Clinical chemistry and hematology profiles of the aging rat. Lab Anim 1992;9:32-45.

Smith D, O'Brien M, Palmer V, Sadowski J. The selection of an adjuvant emulsion for polyclonal antibody production utilizing a low-molecular-weight antigen in rabbits. Lab Anim Sci 1992;42:599-601.

Smith D, Rizzone G. An institutional model for an occupational health program for animal users. Canadian Association for Laboratory and Animal Science Newsletter 1992;26:77-80.

Division of Scientific Computing

The Division of Scientific Computing provides general purpose computing timesharing, networking, desktop computer support, education and training, and database services to the HNRC computing community. The timeshare system supports a large set of biomedical data management and analysis software including SAS and SPSS. Also supported are Systems Analysis Corporations's CLINICAL software for management of data in the Nutritional Evaluation Laboratory, and EXPERT/EASE from Waters Corporation managing 5 HPLC stations. The Division provides networking services internally, by integrating personal computers with the timeshare system and to each other via a 10Base-T Ethernet network, and externally, by providing links with Tufts University via a fiber optic link to the Tufts Ethernet backbone. Internet access is provided through the Tufts University link to the New England Academic and Research Network (NearNet), an Internet provider. TCP/IP based servers and/or clients for standard Internet services (e-mail, FTP, Web, Telnet, etc.) are available on all platforms.

Macintosh and DOS/Windows PCs are used for wordprocessing, statistical analysis, presentation graphics, data management and databases. Scientific Computing supports a set of applications on both platforms. In addition, the Division offers a continuing series of educational and training classes designed to facilitate self-sufficiency in computer and network usage among HNRC technical staff and to provide the necessary skills which allow laboratories and departments to take full advantage of the available computing resources. Training is offered for timeshare systems, as well as for desktop computers, Internet related skills, and various platform independent applications. For tasks beyond the capabilities of individual units, Scientific Computing provides customized consulting, systems analysis and programming services. For the development of special purpose software, there are a set of programming languages, database management tools, forms utilities and a fourth generation application development environment available on several platforms.

SCIENTIFIC COMPUTING STAFF

Pamela Miller, B.S.
Manager, Scientific Computing

Manages Scientific Computing service. Plans, develops and coordinates new computing and information systems.

James K. Prater, B.S., Senior Systems Programmer
Yevgeniya (Jane) Mitkityankaya, B.S., Programmer/Analyst
Bonnie Myers, User Support/Programmer Analyst
Michele Farrell, B.F.A., Staff Assistant

Mass Spectrometry Laboratory

The Mass Spectrometry Laboratory provides the capability to examine the human metabolism of proteins, glucose, triglycerides and other organic macronutrients in response to diet. The methods employ tracers labeled with stable isotopes such as ^{2}H, ^{18}O, ^{13}C and ^{15}N. The laboratory allows HNRC researchers the ability to determine both body composition, based on total body water determinations, and total energy expenditure, using the doubly labeled water method. These services enable scientists to conduct stable isotope studies in human volunteers to determine protein turnover, monitor vitamin metabolism, and to assess turnover and pool sizes of vitamins in the elderly. Laboratory staff assist HNRC investigators in developing methods for the identification and structural characterization of human metabolites.

The facility consists of two laboratories, the first housing a VG SIRA 10 gas isotope ratio mass spectrometer (IRMS), and the second containing an HP 5988A Mass Spectrometer (GC/MS). The IRMS is equipped with a 20 port manifold for direct analysis of water samples that have been reduced to hydrogen gas using zinc. This inlet allows the instrument to measure H/D isotope ratios. The IRMS is also equipped with a 24 port shaker bench for CO_2 equilibration analysis of water samples which allows the instrument to measure $^{16}O/^{18}O$ isotope ratios. The GC/MS lab is equipped with an HP 5890 gas chromatograph interfaced to the mass spectrometer. The GC/MS instrument is operated by HNRC scientists after they are trained by Mass Spectrometry laboratory staff.

MASS SPECTROMETRY STAFF

Gregory G. Dolnikowski, Ph.D.
Laboratory Chief/Scientist II

Provides managerial and scientific direction. Advises scientific personnel on the use of isotope ratio mass spectrometry and gas chromatography/mass spectrometry methods.

Ding Vu, B.S., Research Assistant

SELECTED RECENT PUBLICATIONS

Millar JS, Lichtenstein AH, Cuchel M, Dolnikowski GG, Hachey DL, Cohn JS, Schaefer EJ. Impact of age on the metabolism of VLDL, IDL, and LDL apolipoprotein B-100 in men. J Lipid Res 1995;36:1155-67.

Castaneda C, Dolnikowski GG, Dallal GE, Evans WJ, Crim MC. Protein turnover and energy metabolism of elderly women fed a low-protein diet. Am J Clin Nutr 1995;62:40-8.

Wang X-D, Russell RM, Marini RP, Tang G, Dolnikowski GG, Fox JG, Krinsky NI. Intestinal perfusion of ß-carotene in the ferret raises retinoic acid level in portal blood. Biochim Biophys Acta 1993;1167:159-64.

Tang G, Dolnikowski GG, Blanco MC, Fox JG, Russell RM. Serum carotenoids and retinoids in ferrets fed canthaxanthin. J Nutr Biochem 1993;4:58-63.

Dolnikowski GG. Isomer differentiation by charge inversion tandem mass spectrometry: an investigation into the structure of the ionic products from an S_N(ANRORC) reaction. J Am Soc Mass Spectrom 1992;3:467-70.

Dolnikowski GG, Gross ML, Cavalieri EL. Isomer differentiation in 7,12-dimethylbenz[*a*]anthracene-pyridine adducts by fast atom bombardment tandem mass spectrometry. J Am Soc Mass Spectrom 1991;2:256-8.

Metabolic Research Unit

The Metabolic Research Unit (MRU) supports research protocols utilizing healthy volunteers recruited principally from the metropolitan Boston area. The 25,419 square foot unit includes individual quarters for 14 resident volunteers, a complete metabolic kitchen which services both resident and free-living volunteers, dining room, medical examination rooms, a medical records library, swimming pool and other recreation areas.

Professional recruiters within the Volunteer Services Department identify potential human subjects for research studies through various methods such as newspaper and radio advertising, public service announcements, direct mail, tours and presentations. A registered nurse evaluates all medical history applications for initial qualification. Admissions staff schedule medical screenings and study admissions. This group issues volunteer stipends, maintains a computerized database of scheduling information and provides traditional medical record services.

Research nurses and the Nursing Department's support staff are responsible for implementing research protocols. Integral to this process is the ongoing assessment and monitoring of the physiological and psycho-social status of both the free-living and resident volunteer. HNRC physicians obtain medical histories, conduct physical examinations and provide medical care. Routine blood work, urinalyses, electrocardiograms and X-ray examinations are incorporated as part of the screening process. Physician coverage is in effect 24-hours a day to respond to the medical and psychological needs of resident volunteers.

Registered dietitians and support staff in the Nutrition Services Department are responsible for the interpretation, implementation and successful outcome of the dietary component of research protocols. The Metabolic Nutrition Laboratory is designed and equipped to support metabolic research studies requiring precise nutrient control including the weighing of all foods to the nearest gram, selecting a limited range of high quality, nutritious foods and following precise preparation and serving procedures.

ADMINISTRATIVE STAFF

Robert M. Russell, M.D. Director, Human Services	Directs human studies effort which encompasses activities of the Metabolic Research Unit, the Nutritional Evaluation Laboratory and the Dietary Assessment Unit.
Judith Frazier, R.N., M.Ed. Manager/Director, Nursing	Coordinates and manages daily MRU operation. Plans and directs the multidisciplinary functions and responsibilities of medical, nursing, nutrition and volunteer services. HNRC representative to the Tufts Human Investigation Review Committee.
Marilyn Regan, B.A., Staff Assistant	

NURSING STAFF

Madelyn Hackett, R.N., B.F.A., Head Nurse
Angela Branon, R.N., Staff Nurse
Mary Beth Doherty, R.N., Staff Nurse
Judy Laugherty-Manschreck, R.N., B.S., Staff Nurse
Barbara Maxwell, R.N., B.S.N., Staff Nurse

Jean McShea, R.N., Staff Nurse
Ann Muchowski, R.N., Staff Nurse
Carol Nelsen, R.N., B.S., Staff Nurse
Carol Pergola, R.N., B.S., Staff Nurse
Marie Merchant, Nursing Assistant Certificate, Nursing Assistant
Margaret Mulkerin, Nursing Assistant
Bennette Williams, Nursing Assistant Certificate, Nursing Assistant
Lorna Nichols, Nursing Assistant
Charlotte Earner, Secretary

NUTRITION SERVICES STAFF

Patricia Engel, M.S., R.D., Manager
Helen Rasmussen, M.S., R.D., Research Dietitian
Mazie McIntosh, Sanitation in Food Services/Food Services Management Certificate, Food Production Technician
Verona Bembridge, Food Service Supervisor Certificate, Senior Nutrition Technician
Reety Chauhan, Food Service Coordinator
Shelia Farrell, B.S., Senior Nutrition Technician
Mary Grossman, Nutrition Technician
Shirley Hines, Nutrition Technician
Debra Squillante-Gillis, B.S., Nutrition Technician
Jon B. Nelson, Nutrition Aide
Josephine Zanotti, Portioning Aide
Marie Hedouville, Food Service Worker
David Saia, Food Service Worker

VOLUNTEER SERVICES STAFF

Paula Murphy-Gismondi, M.S.W., Manager
Amy McCall, B.A., Recruiter
Marlaine Parker, Staff Assistant
Arlene Tenney, R.N., B.S., Admissions Coordinator
Faith McDonald, B.A., Admissions Assistant
Carol Quinlan, Admissions Assistant

SELECTED RECENT PUBLICATIONS

McGehee MM, Johnson EQ, Rasmussen HM, Sahyoun N, Lynch MM, Carey M, Massachusetts Dietetic Association. Benefits and costs of medical nutrition therapy by registered dietitians for patients with hypercholesterolemia. J Am Diet Assoc 1995;95:1041-3.

Castaneda C, Charnley JM, Evans WJ, Crim MC. Elderly women accommodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. Am J Clin Nutr 1995;62:30-9.

Hughes VA, Fiatarone MA, Ferrara CM, McNamara JR, Charnley JM, Evans WJ. Lipoprotein response to exercise training and a low-fat diet in older subjects with glucose intolerance. Am J Clin Nutr 1994;59:820-6.

Dawson-Hughes B, Harris S, Kramich C, Dallal G, Rasmussen HM. Calcium retention and hormone levels in black and white women on high- and low-calcium diets. J Bone Miner Res 1993;7:779-87.

Meydani SN, Lichtenstein AH, Cornwall S, Meydani M, Goldin BR, Rasmussen H, Dinarello CA, Schaefer EJ. Immunologic effects of National Cholesterol Education Panel Step-2 diets with and without fish-derived N-3 fatty acid enrichment. J Clin Invest 1993;92:105-13.

Rasmussen HM, Dallal GE, Whelan E, Russell RM. Serum concentrations of retinol and retinyl esters in adults in response to mixed vitamin A and carotenoid containing meals. J Am Coll Nutr 1991;10(5):460-5.

Harris L, Rasmussen HM. Federal nutrition standards. In: Effective menu planning for the elderly nutrition programs. Chicago: American Dietetic Association, 1991:8-20.

Roberts SB, Young VR, Fuss P, Fiatarone MA, Richard B, Rasmussen H, Wagner P, Joseph L, Holehouse E, Evans WJ. Energy expenditure and subsequent nutrient intakes in overfed young men. Am J Physiol 1990;28: R461-9.

Nutrition Evaluation Laboratory

The Nutrition Evaluation Laboratory (NEL) provides clinical and specialized biochemical analyses for HNRC human and animal research studies and for epidemiological and field studies. The laboratory is organized into three main functional units: the Specimen Processing Unit, Clinical Core Unit, and Specialized Chemistries Unit. NEL supported research protocols allow HNRC investigators to perform human studies which complement their basic laboratory studies or conduct large studies which would otherwise not be feasible within their own research programs.

The Specimen Processing Unit (SPU) is responsible for receipt, processing, storage, and tracking of blood, urine, and fecal samples per requirements of the research protocol. SPU personnel prepare protocol-specific urine and blood collection vessels for use by the MRU nursing staff and log test requests for each human subject into the NEL's Laboratory Information Management System.

The Clinical Core Unit (CCU) is licensed by the Federal Government and Commonwealth of Massachusetts to provide clinical results for approximately 30 different procedures related to hematology, blood chemistries, and urinalysis - tests routinely performed on prospective research volunteers. The NEL adheres to strict quality control measures by using commercial reference materials and by participating in the College of American Pathologists' EXCEL external proficiency survey.

The Specialized Chemistries Unit (SCU) provides approximately 80 specialized laboratory procedures on a protocol-specific basis. These procedures are evaluative in nature or generate protocol-specific data from subjects admitted to a research study. Quality control of the esoteric procedures is monitored by using National Institute of Standards and Technology Reference Materials or by commercially available serum and urine quality control materials.

The NEL's Laboratory Information Management System is a DEC VAX based clinical laboratory software package which has been modified for use in the research environment. The system provides long term storage and archiving of 99,000 specimen identification numbers and includes on-line quality control checking, workload reporting, and direct transfer of test results from the Cobas centrifugal analyzer to a subject's medical record files. Study results are available to HNRC investigators in hard copy report formats or by direct electronic transfer into an RS1 table allowing investigators to readily perform statistical and graphical analyses of their studies without manually re-entering numerical or textual results.

NUTRITION EVALUATION LABORATORY STAFF

Gayle Perrone, M.B.A. Manager	Provides managerial and scientific leadership. Assists in research protocol design, development and implementation. Develops methods for nutri-

tional status assessment. Monitors and projects workloads. Establishes and oversees quality control.

Elias Seyoum, PhD., Research Associate
Bella Gindelsky, B.S., Research Assistant
Linda Scott, B.S., R.N., Research Assistant
Bonnie Souppa, M.T., A.S.C.P., Medical Technologist
Irene Ellis, B.S., Senior Research Technician
Shahin Sarkarati, B.S., Senior Research Technician
Scott Valliere, B.S., Research Technician
Diane Paradis, Specimen Processing Technician
Trudy Hedrick, Staff Assistant

SELECTED RECENT PUBLICATIONS

Smith D, Asmundsson G, Perrone G, Scott L, Taylor A. The osteogenic disorder shionogi (ODS) rat - A new model for the study of vitamin C metabolism. CALAS/ACTAL 1995;29:21-3.

Saltzman JR, Kowdley KV, Pedrosa MC, Sepe T, Golner B, Perrone G, Russell RM. Bacterial overgrowth without clinical malabsorption in elderly hypochlorhydric subjects. Gastroenterology 1994;106:615-23.

Jacques PF, Sulsky SI, Perrone GA, Schaefer EJ. Ascorbic acid and plasma lipids. Epidemiology 1994;5(1):19-26.

Bell IR, Morrow FD, Read M, Berkes S, Perrone G. Low thyroxine levels in female psychiatric inpatients with riboflavin deficiency: implications for folate-dependent methylation. Acta Psychiatr Scand 1992;85:360-3.

Scott L, Morrow FD. Clinical chemistries. In: Hartz SC, Russell RM, Rosenberg IH, eds. Nutrition in the elderly: the Boston Nutritional Status Survey. London:Smith-Gordon and Company Limited, 1992;209-31.

Bell IR, Morrow FD, Edman JS, Marby S, Mirages S, Perrone G, Kayne HL, Cole JO. B-Complex vitamin patterns in geriatric and young adult inpatients with major depression. J Am Geriatr Soc 1991;39(3):259-67.

Nutrition Information Office

The Nutrition Information Office (NIO) maintains a database of all manuscripts which have been submitted for publication and entered into the USDA Agricultural Research Service's Research Management Information System. A comprehensive archives of HNRC-supported scientific publications is maintained in this office. This department is responsible for producing HNRC informational materials; developing, coordinating and reporting HNRC continuing education activities and informational programs; and colloaborates with the Division of Scientific Computing on maintaining and identifying appropriate resource links to the HNRC web site *(http://www.hnrc.tufts.edu)*.

In addition, the NIO offers HNRC scientific staff and students access to a multidisciplinary collection of 60 current scientific journals, 15 newsletters, and over 900 books and references pertaining to the biomedical aspects of nutrition and aging. Resources include the personal collection of former Tufts President and renowned nutritionist Dr. Jean Mayer. The NIO occupies 1,250 square feet of space, providing a quiet area for reading and studying. Borrowing privileges for non-reference materials are exclusive to HNRC staff.

The Tufts University's Library Information Processing System (TULIPS) can be accessed from terminals or personal computers throughout the HNRC or off campus. This resource provides access to information available within the Tufts University libraries and on the Internet. In addition, HNRC staff have the capability to search the OVID databases: MEDLINE, BIOSIS, HEALTH and CINAHL.

NUTRITION INFORMATION OFFICE STAFF

Kathleen L. Cappellano, M.S., R.D.
Nutrition Information Coordinator

Manages department services and resources. Press liasion; furnishes news releases, responds to public and media information inquiries. HNRC representative to the Tufts University Health Sciences Library Information and Resources Committee and the Tufts University Library Systems Council.

Gerald Norris, B.A., Staff Assistant

SELECTED RECENT PUBLICATIONS

Cappellano KL, *Assistant Editor,* Rosenberg IH, ed. Nutritional assessment of elderly populations: Measure and function. Proceedings of the Thirteenth Annual Bristol-Myers/Squibb Mead Johnson Nutrition Research Symposium. New York:Raven Press, Ltd., 1995:314 pp.

Cappellano KL. A multi-faceted approach to dissemination nutrition information. J Am Diet Assoc 1994;94 (Suppl):A-83.

Cappellano KL. Who may need supplements? Environmental Nutrition 1991;14(9):1, 6-7.

Cappellano K. Winning at the weight loss game: choosing the right program. Environmental Nutrition 1990; 13(12):3-5.

RESEARCH PROGRAM DESCRIPTIONS

ANTIOXIDANTS RESEARCH LABORATORY

CRIS: Dietary Antioxidants, Aging, and Oxidative Stress Status

MISSION

To understand the role of dietary antioxidants and other environmental factors on oxidative stress status during the aging process. To elucidate age-related changes in antioxidant nutrient requirements for health promotion and disease prevention. The mission is pursued by exploring the effects of vitamins C and E, carotenoids, glutathione, and a variety of phytochemical antioxidants as well as factors such as exercise and xenobiotics on free radical reactions and pathology. Animal models, cell culture, and human volunteers are employed in this research program.

INVESTIGATORS

Jeffrey Blumberg, Ph.D. Laboratory Chief/Senior Scientist Professor, Nutrition	Provides overall direction to the laboratory. Develops integrated research programs.
Roderick Bronson, D.V.M. Scientist I Associate Professor, Pathology	Directs studies on biomarkers of aging and dietary restriction.
Garry Handelman, Ph.D. Scientist II Assistant Professor, Nutrition	Directs studies of antioxidants and other factors on oxidative stress and aging.
Ruth Lipman, Ph.D. Scientist II	Collaborates on studies of biomarkers of aging in animal models.

VISITING SCIENTISTS

Hajiya Abasi, M.D.
Takayuki Ikeda, Ph.D.

TECHNICAL SUPPORT

Zachary Nightengale, B.S., Research Technician

Andrew Halpner, M.S., Graduate Research Assistant
Vincent Tang, M.S., Graduate Research Assistant

CURRENT PROJECTS

- Effect of black tea on biomarkers of oxidative stress status and risk of colorectal cancer in healthy older adults.
- Indices of antioxidant defense and oxidative stress status in brain samples obtained from Alzheimer's disease patients and control subjects.
- Effect of antioxidant interventions on oxidant damage to proteins including protein carbonylation reactions.
- Examination of the role of vitamin C in recycling vitamin E rat hepatocytes *in vitro*.
- Effect of vitamin E, flavonoids, and fish oil on degenerative renal disorders in the rat.
- Effect of oxidative stress status and antioxidants on NFκB modulation of vascular cell adhesion molecule gene transcription and expression in human aortic endothelial cells *in vitro*.
- Effects of vitamin E supplementation on moderate and high intensity exercise in young and older women.

RECENT RESEARCH ACCOMPLISHMENTS

Long-term vitamin E supplementation beneficially influences lipid peroxidation and immune function in young and older adults. Vitamin E supplementation increased α-tocopherol and decreased α-tocopherol and lipid peroxide levels in the plasma of healthy young and older adults participating in a six-month double-blind, placebo-controlled clinical trial. Vitamin E supplementation significantly increased cellular immune responses assessed via delayed-type hypersensitivity skin testing in both age groups, although this effect was greater in the older adults.

Vitamin E protects against exercise-induced oxidative damage in young and older men. The vitamin E content of muscle decreased following a bout of intense eccentric exercise. In a double-blind, placebo-controlled study in healthy sedentary men, vitamin E supplementation for seven weeks reduced elevations in muscle conjugated dienes and urinary thiobarbituric acid reactive substances following the exercise. Vitamin E also restored acute phase immune responses, e.g., neutrophilia and myocellular enzyme efflux, in older men to levels found in young subjects and significantly influenced cytokine production.

Vitamin E requirements are increased by fish oil supplementation. An analysis of commercial fish oil supplements revealed over a three-fold difference in vitamin E content. Healthy women consuming fish oil supplements for three months had elevated concentrations of circulating lipid peroxides. Fish oil supplements do not contain sufficient vitamin E to protect against increased rates of lipid peroxidation associated with large intakes of n-3 polyunsaturated fatty acids.

Beta-carotene supplementation increases tissue concentrations and the antioxidant capacity of plasma in older women. Long-term ß-carotene supplementation of middle-aged and older men participating in the Harvard Physicians Health Study showed a significant elevation of ß-carotene in plasma as well as in red and white blood cells. Despite the increases in RBC and WBC total carotenoid concentration remained unchanged. *In vitro* tests of samples taken from healthy older women participating in a double-blind, placebo-controlled trial of ß-carotene supplementation revealed increases in lag phase and resistance of plasma phosphotidylcholine to peroxidation

Dietary fat affects plasma phospholipid hydroperoxides. Volunteers participated in a clinical trial consisting of successive five week periods during which they consumed beef tallow, corn oil or olive oil as two-thirds of their dietary fat. Plasma phosphotidylcholine hydroperoxide levels in the subjects were significantly lower following the olive oil and beef tallow diets than following corn oil consumption.

Vitamin E protects human aortic and venal endothelial cells from oxidative stress. Human endothelial cells in culture absorbed vitamin E from the media in a dose-dependent fashion. Increased concentrations of vitamin E diminished hydrogen peroxide production under normoxia as well as following simulated ischemia-reperfusion conditions and protected cell viability against oxidized low density lipoproteins.

Caloric restriction alters liver glutathione and reduces the incidence of age-related pathologies in laboratory animals. Caloric restriction reduced the age-associated decline in liver glutathione seen in mice fed *ad libitum*. The rate of occurrence of all types of lesions in 20 inbred and hybrid genotypes of mice and rats fed diets restricted in calories displayed significant reductions in neoplasms and other lesions relative to *ad libitum* controls. Variations in the incidence and type of lesions noted between the genotypes, sexes, and ages of the animals in both groups suggest that a characterization of lesions may serve as a biomarker of aging.

Glutathione monoethyl ester delays the onset of cataracts in rats. Rats fed increasing dietary concentrations of galactose demonstrated a dose-related increase in the prevalence and severity of cataracts. Glutathione monoethyl ester administered intraparenterally during the dietary treatment increased tissue glutathione concentrations and reduced the rate of cataractogenesis. Dietary glutathione did not affect the development of cataracts in Emory mice.

Fish oil supplementation increases lipid peroxides and the requirement for vitamin E in rats. Rats fed fish oil diets maintained lower plasma and tissue levels of vitamin E than those that received corn or coconut oil. Significant increases in vitamin E intake were necessary to prevent the decline. The absorption of ^{14}C α-tocopherol into mesenteric lymph decreased ten-fold after oral administration with fish oil relative to corn oil.

Chronic ethanol consumption adversely affects vitamin E status in rats. Feeding rats a diet containing ethanol decreased α- and increased α-tocopherol in hepatic lipids and induced a fatty liver similar to that observed in alcoholics. Reduction of hepatic antioxidant defenses and increases in lipid peroxidation may contribute to the pathogenesis of alcohol-induced liver disease. Alcohol intake also affected tocopherol metabolism in lung and testes. Relative to young rats, old animals had a slower rate of alcohol elimination and lower hepatic ethanol- and acetaldehyde-metabolizing enzyme activities which were associated with greater susceptibility to alcohol toxicity.

SELECTED RECENT PUBLICATIONS

ARL-J196 Martin A, Zulueta J, Blumberg JB, Hassoun P, Meydani M. Effect of vitamin E on hydrogen peroxide production by human vascular endothelial cells after hypoxia/reoxygenation. Free Radic Biol Med 1996;20:99-105.

ARL-J296 Lipman RD, Chrisp CE, Hazzard DG, Bronson R. Pathologic characterization of Brown Norway, Brown Norway x Fischer 344, and Fischer 344 x Brown Norway rats with relation to age. J Gerontol 1996;51A:B54-B59.

ARL-C196 Blumberg JB. Status and functional impact of nutrition in older adults. In: Schneider EL, Rowe JW, eds. Handbook of the biology of aging, Vol. V. Orlando, FL:Academic Press 1996:393-414.

Taylor A, Lipman RD, Jahngen-Hodge J, et al. Dietary calorie restriction in the Emory mouse: Effects on lifespan, eye lens cataract prevalence and progression, levels of ascorbate, glutathione, glucose, and glycohemoglobin, tail collagen breaktime, DNA and RNA oxidation, skin integrity, fecundity, and cancer. Mech Ageing Dev 1995;79:33-57

Lipman RD, Smith DE, Bronson RT, Blumberg JB. Is late-life caloric restriction beneficial? Clin Exp Res 1995;7:136-9.

Cannon JG, Fiatarone MA, Meydani M, Gong J, Scott L, Blumberg JB, Evans WJ. Aging and dietary modulation of elastase and interleukin-1ß secretion. Am J Physiol 1995;37: R208-R213.

Mune M, Meydani M, Jahngen-Hodge J, Martin A, Smith D, Palmer V, Blumberg JB, Taylor A. Effect of calorie restriction on liver and kidney glutathione in aging Emory mice. AGE 1995;18:43-50.

Jacques PF, Halpner AD, Blumberg, JB. Influence of combined antioxidant nutrient intakes on their plasma concentrations in an elderly population. Am J Clin Nutr 1995;62:1228-33.

Blumberg JB. Consideration of the scientific substantiation for antioxidants vitamins and ß-carotene in disease prevention. Am J Clin Nutr 1995;62(Suppl):S1521S-6S.

Blumberg JB. The requirement for vitamins in aging and age-associated degenerative conditions. Bibl Nutr Dieta 1995;52:108-15.

Blumberg JB. Vitamin E beneficially modulates immunes responses and risk for chronic diseases. In: Urano S, ed. Proceedings of the Fifth Annual Society of Vitamin E Science. Tokyo:Japan Scientific Societies Press, 1995:1-11.

Blumberg JB, Meydani M. The relationship between nutrition and exercise in older adults. In: Gisolfi CV, Nadel ER, Lamb DR, eds. Perspectives in exercise science and sports medicine, Vol. 8. Carmel, IN: Cooper Publishing Group, 1995:353-93.

Halpner AD, Blumberg JB. Assessment of antioxidant vitamin status in older adults. In: Rosenberg IH, ed. Nutritional assessment of elderly populations: measure and function. New York: Raven Press, 1995:147-65.

Blumberg JB. Interactions between vitamin E, free radicals, and immunity during the aging process. In: Armstrong D, ed. Free radicals in diagnostic medicine. New York: Plenum Publishing Corp. Adv Exp Med Biol 1994;366:325-33.

Meydani M, Evans WJ, Handelman G, Biddle L, Fielding RA, Meydani SN, Burrill J, Fiatarone MA, Blumberg JB, Cannon JG. Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. Am J Physiol 1993;264 (Regulatory Integrative Comp Physiol 33):R992-8.

Meydani M, Meydani SN, Blumberg JB. Modulation of dietary vitamin E and selenium of platelet thromboxane A2 and aortic prostacyclin synthesis in rats. J Nutr Biochem 1993;4:322-6.

Gogu SR, Blumberg JB. Vitamin E increases interleukin-2 dependent cellular growth and glycoprotein glycosylation in murine cytotoxic T-cell line. Biochem Biophy Res Commun 1993;193:872-7.

Bronson RT, Lipman R, Harrison D. Age-related gliosis in the white matter of mice. Brain Res 1993;609:124-8.

Bronson RT, Lake BD, et al. Motor neuron degeneration (Mnd) of mice is a model of neuronal ceroid lipofuscinosis (Batten disease). Ann Neurol 1993;33:381-5.

Lipman R, Gaillard ET, Harrison D, Bronson RT. Husbandry factors and the prevalence of age-related amyloidosis in mice. Lab Anim Sci 1993;43:439-43.

Blumberg JB. Nutritionally related problems in the elderly. In: Macrae R, ed. Encyclopaedia of Food Science, Food Technology & Nutrition. London: Academic Press, Ltd., 1993:1543-8.

Gogu SR, Blumberg JB. Vitamin E enhances murine natural killer cell cytotoxicity against YAC-1 tumor cells. J Nutr Immunol 1992;1:31-8.

Meydani M, Blumberg JB. Vitamin E. In: Hartz SC, Russell RM, Rosenberg IH, eds. Nutrition in the elderly: the Boston Nutritional Status Survey. London: Smith-Gordon and Company Limited, 1992:103-9.

BODY COMPOSITION LABORATORY
and
Sarcopenia Research Program

CRIS: Energy Regulation and Body Composition in Aging

MISSION

To evaluate the effect of nutrition on the dynamic interactions between the body's protein, water, fat, and bone and to study the relationship of these changes to the process of aging. The laboratory includes four principal facilities: Whole Body Counter; Partial Body and Small Animal Counter; Neutron Activation Facility; and Neutron Generator Facility for the *in vivo* measurement of fat. To understand the mechanisms leading to loss of muscle mass with age, and develop appropriate interventions that reverse this decline. To develop new techniques of measuring muscle mass accurately in vivo. To determine the role of hormonal and immunologic mediators in the development of sarcopenia of aging, using in vitro, animal, and human studies of aging as well as in appropriate disease models of cachexia. Such models include rheumatoid arthritis and congestive heart failure, among others. To assess the role of diet, physical activity, and specific cytokine or hormonal interventions in the prevention and reversal of sarcopenia.

INVESTIGATORS

Joseph J. Kehayias, Ph.D.
Laboratory Chief/Scientist I

Leads research and develops new approaches to measure body composition and radioisotope kinetics.

Ronenn Roubenoff, M.D., M.H.S.
Scientist I, Sarcopenia Research Program
Assistant Professor, Medicine and Nutrition

Conducts research in body composition and cytokine production in human subjects and animal models.

Lisa Freeman, D.V.M, Ph.D.
Scientist III, Sarcopenia Research Program

VISITING SCIENTISTS

Aminah Jatoi, M.D.

TECHNICAL SUPPORT

Ding Vu, B.S., Research Assistant
Leslie Abad, B.S., Senior Research Technician
Nancy Lundgren, B.S., Research Technician
En-Pei Chiang, M.S., Graduate Research Assistant
Yu-ling Yang, B.S., M.S.P.H., Graduate Research Assistant
Trudy Hedrick, Staff Assistant

CURRENT PROJECTS

- Effect of age and inflammatory cytokines on regulation of protein metabolism and body composition in humans.
- Study of lean tissue depletion with aging.
- Development of techniques for measuring fat distribution patterns *in vivo* in humans.
- Development of a rat model of lean body mass depletion with chronic inflammatory stimulation to determine whether inflammation leads to premature aging.
- Improving techniques for measuring body composition in elderly populations.

RECENT RESEARCH ACCOMPLISHMENTS

New models for the determination of total body fat promise more accurate body composition assessment. Because of the high carbon content of triglycerides, total body carbon (TBC) measurements provide the most direct way of evaluating body fat. Body fat and protein are the main contributors to TBC, while bone ash and carbohydrates contribute less then 3 percent. The C-K model is based on the measurements of TBC and total body potassium (TBK). Protein is estimated from TBK and TBC is corrected for the carbon contribution due to protein and glycogen. This model was validated against the Carbon-Nitrogen-Calcium model, the most direct method for measuring body fat, where all the contributions to TBC are measured [Total Body Nitrogen (TBN) for protein and glycogen and Total Body Calcium (TBCa) for bone ash]. The Hydrogen-Water Model is based on the partition of total body hydrogen to its contributing compartments. Body water (TBW) and fat are the main contributors to total body hydrogen (TBH), followed by protein which contributes less than 10 percent. Therefore, a TBH measurement can be associated with the body's fat after it is corrected for the contributions of TBW and protein. Unlike the C-K model, the accurate measurement of both TBH and TBW are essential for a reasonable measurement of body fat. Protein, however, a minor contributor to TBH, can be estimated indirectly. The validity of this model was tested against the Carbon-Nitrogen-Calcium model. This new approach to body composition relies on the partition of a major element (carbon or hydrogen) to its contributing compartments. These models have the advantage of being independent of assumptions on the composition of lean tissue and thus qualify for studies of individuals of any age and health condition.

The effect of aging on lean body mass and fat. Understanding the mechanisms which govern the depletion of lean body mass with age may suggest a way of preserving lean tissue and functional capacity, and consequently the quality of life of the very old. In order to measure the differences in each body compartment as a function of age, a cross-sectional study is being conducted in healthy volunteers aged 20-100 years. In aging, the assumptions of indirect body composition techniques -- such as the "constant" hydration coefficient of lean body mass or the "constant" density of fat free mass -- may break down, presenting a similarity between studying normal aging and some catabolic diseases. Therefore, body composition techniques which do not make assumptions about the composition of lean tissue have been selected. Body fat, water and cellular mass are assessed "directly" by neutron inelastic scattering, dilution of tritiated water and total body K-40 (TBK) measurements. Results show a dramatic decline in cellular mass after the age of 70. Although TBK is systematically higher in males, there is no significant sex dependence on the rate of TBK depletion. A systematic increase in body fat content with age even when body weight is decreased has also been observed.

Understanding how the immune system may control protein metabolism and lead to the loss of lean body mass may provide a better understanding of the same decline seen in aging. Loss of lean mass - especially muscle mass, is one of the major physiologic hallmarks of aging. Based on studies of starvation and acute illness, loss of more than 40 percent of lean mass is fatal. To the extent that aging is an accumulation of environmental "lesions" over time it is attractive to consider lean body mass as an index of the degree of insult suffered by the body with aging. The presence of disease would then cause accelerated aging by increasing the number of insults accumulated during a fixed time. Research has demonstrated exactly such an increased loss of lean mass - with people with rheumatoid arthritis having 15 percent less lean mass than controls matched for age, sex, race, and weight. In addition, subjects with rheumatoid arthritis produced significantly more of the cytokine tumor necrosis factor-α (TNF) than the controls. Because TNF is known to alter protein metabolism and cause catabolism, this suggests that the immune system may

play a role in determining body composition.

***In vivo* measurement of regional body fat in humans using neutron inelastic scattering.** Certain body fat distribution patterns have been associated with increased risk for cardiovascular disease, for both men and women, independently of total body fat. A low flux of pulsed, fast neutrons is used for the simultaneous detection of regional carbon and oxygen *in vivo* in humans. The carbon to oxygen ratio in tissue is a measure of fat content, because of the dramatic difference in elemental composition between fat and lean. The measurement of total body carbon (TBC) has been a tested technique for the estimate of total body fat, after corrections are applied for the contributions to TBC of non-fat tissues such as protein. Because of the technical difficulties of measuring small amounts of nitrogen simultaneously with carbon, this model cannot be applied easily to the measurement of regional body fat,. With the development of a miniature high repetition rate D-T neutron generator and the refinement of neutron-tolerant large bismuth germanate (BGO) detectors, scientists have demonstrated that the regional simultaneous detection of carbon and oxygen can be achieved at low radiation exposure. This development has allowed the detection of TBC at lower exposure and made possible the measurement of regional C/O ratio, and thus regional fat, *in vivo*.

Improvement of methods of measuring body composition in elderly populations. In conjunction with investigators in the Framingham Heart Study, new, specific bioelectrical impedance equations validated against dual-energy x-ray absorptiometry have been developed. These equations, developed in a population aged 70-92, improved the accuracy and precision of bioelectrical impedance dramatically. The impedance method offers great promise for measuring large populations quickly and inexpensively, but requires the development of population-specific equations.

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CALCIUM AND BONE METABOLISM LABORATORY

CRIS: Role of Nutritional Factors in Maintaining Bone Health in the Elderly

MISSION

To examine ways in which diet and nutritional status in combination with exercise and hormones, particularly estrogen and parathyroid hormone, influence age-related loss of bone density. To determine the extent to which increased calcium and vitamin D intake can mitigate bone loss and prevent the development of osteoporosis and spontaneous fractures in the elderly. To examine intestinal adaptation to altered calcium intake in black and white women.This mission is pursued through clinical studies in which the effects of modifying the diet and/or activity level on calcium absorption and bone density are measured in healthy elderly volunteers.

INVESTIGATORS

Bess Dawson-Hughes, M.D. Laboratory Chief/Scientist I Associate Professor, Medicine	Provides leadership to the laboratory. Develops new approaches and methods to measure the relationship between nutrient intake, absorption and bone health.
Elizabeth Krall, Ph.D. Scientist II	Studies effects of heredity and lifestyle on bone mass. Examines the relationships between alveolar

bone loss, tooth loss, and systemic bone loss.

Susan Harris, M.S.
Research Associate

TECHNICAL SUPPORT

Dibby Falconer, M.S., Recruiter/Nutritionist
Eleanor Joyce, Recruiter Assistant
Karen Sachs, B.A., Research Coordinator
William Comer, B.S., Research Technician
Lauren Farina, Research Technician
Carol Kitchenka, B.S., Research Technician

CURRENT PROJECTS

- A double-blind study of the effect of calcium and vitamin D on bone loss in men and women over age 65 years.
- Effect of season on rates of bone loss and body composition in men and women.
- Influence of season on photosynthesis and photodegradation of vitamin D.
- A comparison of the hormonal and calcium absorption responses to calcitriol in black and white women.
- Effects of physical activity on net intestinal absorption and renal handling of calcium in healthy postmenopausal women.
- Evaluation of the usefulness of selected bone mass measurement technologies in the assessment of rates of bone loss in the elderly.
- Longitudinal study of the effect of life style on rates of bone loss in the Framingham cohort.

RECENT RESEARCH ACCOMPLISHMENTS

New approach for defining vitamin D requirements. Currently there is no consensus on how to define the vitamin D requirement of the elderly. To develop a working definition of vitamin D adequacy the relationship between vitamin D intake and serum concentrations of 25-hydroxyvitamin D [25(OH)D] and parathyroid hormone (PTH) in over 300 healthy postmenopausal women was examined. In the late winter, serum 25(OH)D declines and PTH increases. The seasonal increase in PTH is important because this hormone has the potential to accelerate bone loss. The determination of vitamin D intake required to maintain an adequate 25(OH)D level and prevent the wintertime increase in PTH is under investigation. In a double-blind trial in women with adequate calcium intakes, a 400 IU vitamin D supplement retarded bone loss in the winter and provided an overall benefit at the spine. Further investigation is underway to determine whether a higher intake of vitamin D will add benefit or whether the current Recommended Dietary Allowances for vitamin D, 200 IU per day, is sufficient to minimize bone loss.

Diet, sun and serum 25-hydroxyvitamin D levels. In elderly women, the contribution of vitamin D intake to plasma 25-hydroxyvitamin D concentration in the winter was lower in women with high sunlight exposure than in those who spent less time in the sun. *In vitro*, sunlight stimulates photodegradation as well as photosynthesis. This study raises the possibility that vitamin D of both dietary and skin origin may be regulated by sunlight.

Evidence for gut resistance to the action of vitamin D in blacks. Differences between blacks and whites in calcium regulating hormones might provide insight into why blacks have greater bone mass and less osteoporosis. Black and white women display similar levels of calcium absorption. In black women the higher levels of the active vitamin D metabolite that stimulates intestinal calcium transport suggests that blacks may have a gut resistance to the action of activated vitamin D. Higher levels of active vitamin D may promote bone mineralization.

Heritable and life-style determinants of bone density. Familial resemblance in bone density is a composite of genetic similarity and similarity in life-style factors that influence rates of bone mineral gain and loss. In a study of forty families, each consisting of natural parents, a daughter and son, 46 to 62 percent of the variance in bone density was attributable to heredity and the remainder to other factors including environment.

Season influences mood in normal women. A prospective study of mood was conducted in 250 healthy postmenopausal women with no history of psychiatric disorder. Anxiety, depression, anger, and fatigue scores were higher in the fall than in the spring or summer.

Thiazides and seasonal bone loss. Thiazides are known to reduce bone loss and osteoporotic fractures. In 250 healthy postmenopausal women, thiazide use was associated with lower wintertime parathyroid hormone levels and less wintertime bone loss. This effect was not seen in the summer, suggesting that thiazides cause a seasonal reduction in the bone remodeling rate.

Body composition changes with season. Fat, lean, and bone tissue masses change with time of year in healthy postmenopausal women with stable year round weights. In the summer/fall, lean and bone tissue mass increase and fat decreases in the arms, legs, trunk, and whole body. In the winter/spring these changes reverse. Overall, there is a loss of lean tissue mass in the legs and an increase in fat tissue mass in the trunk.

Rates of bone loss vary with age. In a longitudinal study of 288 healthy postmenopausal women, spinal bone loss declined by about 2 percent per year in the first 5 years after menopause, 1 percent per year in women from 6 to 20 years after menopause, and approximately 0.4 percent per year thereafter. At the heel, the rate of loss was 2.5 percent per year just after menopause and declined to 1 percent per year thereafter. Loss from the femoral neck and radius was steady over the range of 1 to more than 20 years after menopause, and averaged about 0.4 percent per year at each site. Trabecular bone is more sensitive than cortical bone to estrogen loss at menopause. Bone loss at all sites continues into old age.

Body weight influences rate of bone loss. In healthy postmenopausal women, weight was inversely related to rate of bone loss from the spine in those up to but not beyond 106 percent of ideal body weight. This finding suggests that thinness is a risk factor for osteoporosis, rather than that obesity protects against bone loss.

Precision of body composition measurements by the dual-energy x-ray absorptiometry measurements. Body composition, by region and overall, can be assessed precisely by dual-energy x-ray absorptiometry. For the regions of the arms, legs, trunk, and whole body, lean tissue can be measured with a coefficient of variation (CV) of 1.0 to 3.3 percent, bone tissue with a CV of 1.1 to 2.6 percent and fat tissue with a CV of 2.2 to 5.5. This method is suitable for detecting small changes in regional and whole body composition.

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Dawson-Hughes B, Harris SS, Finneran S, Rasmussen HM. Calcium absorption responses to calcitrol in black and white premenopausal women. J Clin Endocrinol Metab 1995;80: 3068-72.

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Krall EA, Dawson-Hughes B. Heritable and lifestyle determinants of bone mineral density. J Bone Miner Res 1993;8:1-9.

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ENERGY METABOLISM LABORATORY

CRIS: Energy Metabolism and Body Composition in Aging

MISSION

To examine how body weight is normally regulated and why many people tend to gain weight as they grow older. The importance of genetic and environmental factors in determining body composition and energy regulation, and quantifying optimal dietary energy requirements are under investigation. Research involves studies at the level of whole-body physiology such as examining the importance of energy expenditure and energy intake in determining body fat gain during adult life. In addition, hormonal and cellular investigations are underway to identify the underlying metabolic cause of differences in body composition and energy regulation between people.

INVESTIGATORS

Susan B. Roberts, Ph.D. Laboratory Chief/Scientist I Associate Professor, Nutrition and Psychiatry	Leads research program and develops new techniques.
Andrew S. Greenberg, M.D. Scientist II Assistant Professor, Endocrinology and Molecular Medicine	Investigates the cellular and molecular aspects of energy regulation and adipose tissue.
Edward Saltzman, M.D. Scientist III	Examines the role of inheritance in energy metabolism and its genetic determinants.
Sandra Souza, Ph.D. Research Associate	
Joseph Surrao, M.D., M.P.H. Research Associate	

TECHNICAL SUPPORT

Paul Fuss, B.A., Senior Research Assistant
Sai Das, M.S., Research Assistant
Nicholas Hays, M.S., Graduate Research Assistant
Daniel Hoffman, M.S., Graduate Research Assistant
Angela Vinken, M.S., Graduate Research Assistant
Alberto Corrales, B.A., Research Technician
Gretchen Beherrell, B.A., Office Assistant

CURRENT PROJECTS

- Investigation of energy metabolism and body composition during overfeeding and underfeeding to determine whether various individuals and age-groups have a different susceptibility to weight gain during overeating and weight loss during undereating.

- Determination of the genetic inheritance of body composition and energy regulation in identical twins.

- Establishment of the normal energy requirements of adult men and women using accurate stable isotope techniques.

- Investigation of the roles of key hormonal and cellular parameters in the regulation of energy balance.

- Examination of the changes in energy expenditure with the menstrual cycle and aging in women.

- Development of new and improved techniques for the measurement of energy expenditure and the assessment of dietary compliance in metabolic studies.

RECENT RESEARCH ACCOMPLISHMENTS

Body energy balance appears to be regulated by remarkably sensitive mechanisms in normal-weight individuals. This sensitivity can be illustrated by calculations of the effects of a persistent imbalance between energy intake and expenditure. For example, an imbalance of only 2 percent between energy intake and energy expenditure in a typical young adult male would lead to an energy imbalance of about 40,000 kcal/year, resulting in a body weight gain of nearly 8 lbs. per year and 80 lbs. per decade. The successful regulation of body energy balance which prevents such a substantial change in body energy stores must involve adaptive fluctuations in energy expenditure and/or energy intake over periods of several days or longer. The question of whether energy intake or energy expenditure is the primary factor maintaining energy balance remains a subject of major controversy. The results of an overfeeding study in young men recently conducted in the laboratory provide strong evidence indicating that there is no variability between individuals in the susceptibility to weight gain during overeating when methodological problems are avoided. This research indicates that energy wasting mechanisms are *not* a major feature of energy regulation in humans, and that the control of food intake may be the primary means by which body weight is modulated.

Lean individuals consume and expend significantly more energy to maintain body weight than fatter individuals. Results obtained by employing the new doubly labeled water method to measure the total energy expenditure of healthy young and elderly men leading their usual lives indicate that normal energy requirements are far higher than current recommendations indicate. This new information can be used to define suitable levels of energy intake and expenditure for the prevention and treatment of obesity in men, and to determine appropriate recommendations on average dietary energy needs.

The accuracy of dietary nutrient intakes in metabolic studies. Because continuous observation of research subjects is rarely feasible, there remains much uncertainty over the accuracy of dietary intake data. Two new techniques were developed to objectively monitor subjects' compliance with the dietary requirements of metabolic protocols. The para-aminobenzoic acid (PABA) compliance test involves supplementation of the metabolic diet with para-aminobenzoic acid and measurement of the PABA metabolites in urine collections. This test determines whether the subjects actually consume all the food that is provided to them. The osmole excretion rate technique is based on measurement of the osmolality of urine collections and comparison of the measured urinary osmolar load with the load predicted from dietary protein, sodium and potassium intakes. This method detects whether the subjects consume food in addition to the metabolic diet (when the measured osmolar load will be high). The combination of these two techniques to detect non-compliance on metabolic diets provides investigators for the first time with objective evidence of the accuracy of dietary intake data. Using the techniques, significant non-compliance in overfeeding studies were detected that would not have been found by observation and which would have had a major influence on the study outcome.

SELECTED RECENT PUBLICATIONS

EML-J196 Roberts SB, Fuss P, Dallal GE, Atkinson A, Joseph L, Evans WJ, Fiatarone MA, Greenberg AS, Young VR. Effects of age on energy expenditure and substrate oxidation during experimental overfeeding in healthy men. J Gerontol 1996;51A:B148-57.

EML-J296 Roberts SB, Fuss P, Dallal GE, Heyman MB, Young VR. Effects of age on energy expenditure and substrate oxidation during experimental underfeeding in healthy men. J Gerontol 1996; 51A: B158-66.

EML-J396 Sawaya AL, Tucker K, Tsay R, Willett W, Saltzman E, Dallal GE, Roberts SB. Evaluation of four methods for determining energy intake in young and older women: comparison with doubly labeled water measurements of total energy expenditure. Am J Clin Nutr 1996;63: 491-9.

EML-J496 Sawaya AL, Dallal GE, Solymos G, DeSousa MH, Ventura ML, Roberts SB, Sigulem DM. Obesity and malnutrition in a Shantytown population in the city of Sao Paulo, Brazil. Obesity Research 1996;3 Suppl:107s-15s.

Roberts SB, Dietz W, Sharp T, Dallal GE, Hill JO. Multiple laboratory comparisons of the doubly labeled water technique. Obes Res 1995;3(Suppl 1):3-14.

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Roberts SB, Ferland G, Young VR, et al. Objective verification of dietary intake by measurement of urine osmolality Am J Clin Nutr 1991;54:774-82.

Roberts SB, Heyman MB, Evans WJ, Fuss, P, Young, VR. Dietary energy requirements of young adult men, determined using the doubly labeled water method. Am J Clin Nutr 1991;54: 499-505.

Roberts SB, Young VR, Fuss P, et al. Body weight regulation in young men: effects of overfeeding on energy expenditure and subsequent nutrient intakes. Am J Physiol 1990;259:R461-9.

Egan JJ, Greenberg AS, Chang MK, Londos C. Control of endogenous phosphorylation of the major cAMP- dependent protein-kinase substrate in adipocytes by insulin and ß-adregenic stimulation. J Biol Chem 1990;265:18769-75.

EPIDEMIOLOGY PROGRAM

CRIS: Nutrition, Epidemiology and Aging

MISSION

To identify the determinants of nutritional status in the elderly, to relate nutritional status to health and well-being, to define groups at special risk of nutritional problems, and to evaluate nutritional programs which service the elderly. Current studies address age-associated changes in energy and nutrient intake; constitutional, psychosocial and environmental determinants of food choices; nutritional determinants of neurobehavioral function, and of age-related changes, such as lens opacification.

INVESTIGATORS

David Rush, M.D. Program Director/Senior Scientist Professor, Nutrition and Community Health	Provides leadership to the research program. Develops methods to study the effects of nutritional status and diet on health; nutritional screening in the elderly; and the impact of socioeconomic forces on nutritional status.
Paul F. Jacques, D.Sc. Associate Chief/Scientist I Associate Professor, Nutrition	Examines the effects of vitamin status on age-related decline in cardiovascular and visual function.
Johanna T. Dwyer, D.Sc., R.D. Senior Scientist Professor, Nutrition	Examines the associations of diet with subsequent health especially renal function and body composition. Studies the validity of intake measures and recall accuracy for data pertaining to diet and body weight.
William H. Dietz, M.D., Ph.D. Scientist I Associate Professor, Pediatrics	Investigates the relationships between energy metabolism and body composition and growth patterns and later morbidity/mortality.

Katherine Tucker, Ph.D. Scientist I Associate Professor, Nutrition	Studies dietary methodology, diet and disease relationships, social and behavioral factors related to diet and nutritional status of the elderly, and nutrition and health among Hispanic elderly.
Aviva Must, Ph.D. Scientist II Assistant Professor, Family Medicine, Community Health, and Nutrition	Assesses the influence of early body composition on mortality and morbidity among older persons and the delineation of the role of reproductive factors on the incidence and maintenance of obesity in women.
Karen Riggs, Ph.D. Scientist III	Conducts research on the nutritional effects on cognitive aging and the relationship of personality and cognitive abilities to the accuracy of dietary self-report.
Odilia Bermudez, Ph.D. Research Associate/Project Manager	Examines the health and nutritional status of Hispanic elderly.

TECHNICAL STAFF

Sharron Rich, B.Sc., M.P.H., Data Analyst
Gail Vanca, B.A., Data Analyst
Lisa Bianchi, Programmer Analyst
Dalila Avila, Phlebotomist
Rosaline Bowen, M.A., Staff Assistant

CURRENT PROJECTS

- Investigation of the relationship between vitamin B_{12} and folate status and neurobehavioral function in the Veterans Administration Normative Aging Study (NAS), the East Boston aging study, and the Baltimore Longitudinal Study of Aging (BLSA).

- Sociobehavioral determinants of dietary patterns in the elderly.

- Dietary patterns among elderly populations, including the Boston Nutritional Status Survey (NSS), the Framingham Heart Study (FHS) and the NAS; replicative ability and association with socio-demographic characteristics, health behaviors, nutritional status, and health measures.

- Investigation of the relationship between senile cataract and vitamin C, carotenoids, folate, taurine and homocysteine.

- Assessment of vitamin D levels and dietary intake in the FHS, in relationship to bone health, serum lipid and nutrient levels, and diet patterns.

- Effects of niacin depletion and repletion on cognitive function in older adults.

- The relationship of personality and cognitive abilities to the accuracy of dietary self-report.

- Nutrition and frailty among elderly Hispanics in Massachusetts: diet, disease, disability and health access among a statewide random sample.

- Development of a food frequency questionnaire for use with Hispanic adults in the northeastern United States.

- Longitudinal changes in body habitus among members of the NAS; patterns of change and relationships with health outcomes.

RECENT RESEARCH ACCOMPLISHMENTS

Normative dietary patterns of the American elderly. In the NSS, the NAS, and the FHS, dietary patterns identified by cluster analysis--including groups where subjects consume a larger proportion of the their energy from alcohol; meat and potatoes; and cereal, fruit and milk are remarkably consistent across populations of elderly subjects, while those defined by principal components analysis are not. The cereal, fruit and milk group shows consistently better nutrient intakes and measures of vitamin status than other food groups.

Health and socioeconomic associations of low energy intake. In the NSS 680 Boston area volunteers completed three-day diet records. More than 20 percent reported consuming less than two-thirds of the 1989 RDA for energy, magnesium, vitamin B_6, calcium, and zinc. Variables associated with low micronutrient intake (controlling energy intake, age and gender) included non-white race, low income, low education level, alcohol use, eating alone, greater medication use and denture use. A high fat intake was associated with non-white race and lower education levels.

Dietary intakes among elderly subjects vary with age and education level. In the FHS older men consumed more carbohydrate and less animal protein and caffeine than younger men. Older women consumed more carbohydrate and less polyunsaturated fat and alcohol than younger women. Vitamin intakes were directly correlated with education; with more educated women consuming more nutrient dense foods and their male counterparts consuming more nutrient supplements.

Impact of adolescent obesity on health status. In the Third Harvard Growth Study (HGS) follow-up, an adverse impact of adolescent obesity was found on total and coronary heart disease mortality in males, and compromised health and functional status among both males and females. In related studies, reference standards have been developed from the First National Health and Nutrition Examination Survey for obesity and superobesity by race and for the population aged 60-74 years based on triceps skinfold thickness and body mass index.

Vitamins and vascular disease. Vitamin C supplementation increased HDL cholesterol and apo A-I among individuals with initially low plasma vitamin C levels. Plasma folate, vitamin B_{12} and pyridoxal-5'-phosphate are each independent predictors of plasma homocysteine concentrations. Approximately 30 percent of elderly individuals have elevated homocysteine levels; two-thirds of these cases are associated with inadequate levels of these nutrients.

Vitamin C, cataracts and maculopathy. Women with high vitamin C intake have approximately one-third the prevalence of advanced cataract and one-half the prevalence of age-related maculopathy as women with low intakes. Usual intakes of folate, vitamin E and vitamin C measured by a food frequency correlated strongly with plasma levels of these three nutrients.

Body size correlations. Correlations between perceived and actual body size among middle-aged participants in a longitudinal study of body size and maturation were moderate but significant. Correlations were influenced by gender and phases of physical growth. In general, accuracy of self reports of current body size were not significantly better than recalls of body size up to 50 years earlier. Respondents' recalls of various physiological events was also assessed. Actual and recalled year of menarche were within one year of the actual event in 84 percent of females, 50 percent of both sexes recalled their year of maximal growth in height within a year, and also recalled timing of their maturation equally well. Current Body Mass Index (BMI) was significantly associated with lifetime weight dissatisfaction in both sexes.

B vitamin status may influence cognitive functioning in older adults. Results from two samples from the Boston Veterans Administration Normative Aging Study (NAS) populations indicate that inferior spatial copying and working memory skills may be associated with low B vitamin levels, when scores are adjusted for years of education.

Increase in Body Mass Index with aging. In the NAS over a 15 year period of observation, BMI tended to increase in all but the oldest subjects. Patterns were modified by smoking: continuing smokers remained leaner, while those who stopped smoking gained more weight. Over time, circumference measures tended to increase more than skinfolds, suggesting differential accumulation of central body fat. In the HGS, a 50-year longitudinal study of BMI it was found that BMI before maturity was a poor predictor of mortality in middle age women, but a reasonably good predictor in men. BMI tracking from childhood to middle age was also better in males.

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GASTROINTESTINAL NUTRITION LABORATORY

CRIS: Gastrointestinal Function and Metabolism in Aging

MISSION

To determine how aging and associated factors such as medication use affect the intestinal absorption and metabolism of micronutrients, including carotenoids. Experimental animal and cell culture models, and human volunteers are employed in studies to investigate whether changes in the Recommended Dietary Allowances (RDA) for niacin, vitamin A, vitamin B_2, vitamin B_6, vitamin B_{12} are warranted for the elderly. The chemo-preventive effects of carotenoids against cancer are explored. Research is also conducted in elderly subjects with atrophic gastritis or hypochlorhydria, a significant sub-population of elderly at risk for impaired nutrient absorption and gastric cancer. Perfused intestinal segments and mesenteric lymph cannulae are also used in animal models characterizing the kinetics, energy requirements and age-associated changes in micronutrient uptake and clearance.

INVESTIGATORS

Robert M. Russell, M.D. Associate Director, Laboratory Chief/Senior Scientist Professor, Medicine and Nutrition	Provides overall leadership to the laboratory. Conducts studies on intestinal absorption in elderly subjects with gastric atrophy; the effect of bacterial overgrowth on nutrient bioavailability; and the intermediary metabolism of vitamins and carotenoids as affected by aging.
Norman Krinsky, Ph.D. Senior Scientist Professor, Biochemistry	Investigates mechanisms of carotenoid metabolism by enzymatic pathways and non-enzymatic pathways. Examines role of carotenoids as antioxidants.
Lynne Ausman, D.Sc., R.D. Scientist I Professor, Nutrition	Collaborates on studies of intestinal cholesterol and bile acid metabolism. Studies effect of dietary lipid unsaponifiables on whole body and intestinal metabolism of cholesterol and bile acids.
Elizabeth Johnson, Ph.D. Scientist II Assistant Professor, Nutrition	Investigates vitamin A and carotene absorption and metabolism by the human gastrointestinal tract and peripheral tissues. Studies the chemopreventive actions of ß-carotene against breast cancer.
Judy Ribaya-Mercado, D.Sc. Scientist II Assistant Professor, Nutrition	Examines the effect of aging on water soluble vitamin metabolism and on epithelial cell metabolism of carotenoids.

Guang-Wen Tang, Ph.D. Scientist II	Studies vitamin A metabolism and the effects of UV radiation, medication and disease (e.g., cancer, intestinal bacterial overgrowth) and carotenoid function as chemopreventive agents.
Xiang Dong-Wang, M.D., Ph.D. Scientist II	Develops the ferret as an animal model for studying carotene metabolism. Researches the regulatory factors affecting blood levels of retinoids and the interaction between vitamin E and carotenoids.
Kyung-Jin Yeum, M.D. Research Associate	Conducts studies on metabolism of carotenoids by lipoxagenases.

VISITING SCIENTISTS

John Saltzman, M.D.	Collaborates on human studies of atrophic gastritis and small intestinal bacterial overgrowth. Investigates adherent strains of lactobacilli on lactose tolerance.

TECHNICAL STAFF

Barbara Golner, Senior Research Assistant
Jian Qin, Research Technician
Leisl Castro, M.S., Graduate Research Assistant
Mina-fen Lee, B.S., Graduate Research Assistant
Elmi Tibaduiza, M.S., Graduate Research Assistant

CURRENT PROJECTS

- Vitamin A equivalence of various carotenoids in humans using stable isotopes.
- Determination of the usefulness of carotenoids as therapeutic agents against breast and gastric cancers.
- Detemination of tissue compartmentalization of carotene breakdown mechanisms.
- Gene regulation activity of carotenoic acids.
- Effect of feeding carotenoids and vitamin E on tissue/blood carotenoid and retinoid contents in human blood, fat, buccal epithelium and eye tissues.
- Effect of dietary lactobacilli on small intestinal colonization and lactose digestion in elderly volunteers with and without atrophic gastritis.
- Examination of the effects of small intestinal bacterial overgrowth on intestinal secretion and absorption in subjects with atrophic gastritis.

- Impact of intestinal bacterial populations on vitamin K nutriture.
- Effect of atrophic gastritis and bacterial overgrowth on ethanol metabolism.
- Elucidation of the biological pathways of excentric cleavage of carotenoids via enzyme isolation and gene regulation.
- Development of the ferret as a model to study the effects of carotenoids in gastric cancer prevention.
- Reversal of atrophic gastritis by *Helicobacter pylori* eradication.
- Mechanism of hypocholesterolemic effect of rice bran oil feeding.
- Influence of bile acids on mutagen in colitis-prone cotton-top tamarins.

RECENT RESEARCH ACCOMPLISHMENTS

Excentric cleavage mechanism of beta-carotene exists in the intestines of humans, monkeys, ferrets and rats. Beta-carotene, a vitamin A precursor, is one of the few food components for which there is strong evidence for an anti-cancer role. The mechanism(s) in which ß-carotene may work is uncertain due to a lack in the understanding of its normal metabolism. It has been widely accepted that ß-carotene is converted into two molecules of vitamin A by a central double bond cleavage of ß-carotene by an intestinal enzyme. However, the unequivocal specificity of the enzyme for the central double bond of ß-carotene has not been established. Recent findings provide strong evidence that the same enzyme that primarily cleaves the central double bond of the ß-carotene molecule also cleaves the molecule at several other double bonds resulting in the formation of a mixture of products.

Retinoic acid can be produced from the excentric cleavage of ß-carotene in human intestinal mucosa. How carotenoids are transformed into retinoids is of importance in view of the supposed role of individual retinoids, especially retinoic acid and ß-carotene, in cancer prevention. In *in vitro* and *in vivo* studies the formation of beta apo carotenals and retinoic acid from ß-carotene has been demonstrated. In addition, intermediates such as beta apo-14′ carotenal and beta apo-13′ carotenone have been identified. The formation of retinoic acid from both ß-carotene and beta apo carotenals has been shown to be dose and time dependent. Retinoic acid formation from both beta apo-8′ carotenal and beta apo-12′ carotenal in human intestinal homogenates occurs in the presence of citral, demonstrating that retinoic acid can be produced from the excentric cleavage of ß-carotene via a series of beta apo carotenals as intermediates.

Increased number of bacteria in the stomach and upper small intestine causes poor absorption of food-bound vitamin B_{12}. Impaired absorption of food-bound vitamin B_{12} has been reported in atrophic gastritis, a common condition of aging which is characterized by reduced or no gastric acid output and increased amounts of bacteria in the upper small intestine and stomach. Food-bound vitamin B_{12} is poorly absorbed in atrophic gastritis subjects as compared to normal controls. This poor absorption is reversed by the administration of an antibiotic. The poor digestion of vitamin B_{12} from food protein due to lack of acid seems to play only a minor role in causing the vitamin B_{12} malabsorption. Killing or reducing the number of bacteria in the stomach and upper intestine appears to normalize food-bound vitamin B_{12} absorption.

Gastric emptying is a major modulator of first pass metabolism of alcohol in elderly subjects. Oral intake of ethanol results in lower ethanol blood concentrations when compared to the same dose administered intravenously. This so-called "first pass metabolism" is thought to be attributed to gastric metabolism of ethanol via the enzyme alcohol dehydrogenase. Elderly subjects with and without atrophic gastritis were studied for first pass metabolism before and after antibiotic treatment to reduce the number of upper gastric intestinal bacteria. Gastric emptying rates of an ethanol solution in food were calculated from scintigraphic images. In an additional group of subjects with and without atrophic gastritis, gastric biopsies were obtained for determination of alcohol dehydrogenase activity. Gastric alcohol dehydrogenase activity was found to be significantly lower in the atrophic gastritis subjects versus the controls. However, neither gender nor atrophic gastritis had an effect on first pass metabolism of ethanol in the

elderly. A significant correlation was found between the first pass metabolism of ethanol and gastric half emptying time. These studies are important in elucidating the effects of moderate drinking in elderly humans.

Bacterial overgrowth due to gastric hypochlorhydria is not associated with clinically significant macronutrient malabsorption. Bacterial overgrowth of the small intestine commonly occurs in association with low stomach acid secretion present in atrophic gastritis or during treatment with acid blocking agents such as omeprazole. This study sought to determine the clinical significance of bacterial overgrowth on small intestine absorption and permeability and to evaluate the reliability of non-invasive breath tests to detect bacterial overgrowth in subjects with hypochlorhydria. Seventeen healthy subjects with hypochlorhydria and documented bacterial overgrowth were examined. There was no evidence of fat or carbohydrate malabsorption in any subject. The presence of bacterial overgrowth resulted in higher gut permeability for lactulose and higher amounts of alpha I antitrypsin secretion indicating increased gut leakiness. Lactulose, glucose and C-14 xylose breath tests to detect bacterial overgrowth were not reliable in subjects with hypochlorhydria.

Riboflavin requirement of healthy elderly humans are the same as for young adults. Two groups of riboflavin deficient but otherwise healthy Guatemalan elderly persons over the age of 60 consumed diets varying in fat-carbohydrate ratios. The first group consumed a diet similar in macronutrient content to a low carbohydrate, high fat Western diet. The other group consumed a typical high carbohydrate, low fat Guatemalan diet. It was found that the riboflavin requirement of elderly subjects consuming the Western type diet did not differ from those reported in young adults. However, the requirements of the elderly are influenced by the macronutrient composi-tion of the diet, and higher carbohydrate levels (with a concomitant reduction in fat content) lowers the dietary riboflavin requirement. It is thought that the higher carbohydrate diet causes greater riboflavin synthesis by intestinal bacteria which is subsequently absorbed.

Retinoic acid regulates retinol metabolism via feedback inhibition of retinol oxidation and stimulation of retinol esterification. Retinoic acid has an important regulatory function on gene expression and differentiation, although the mechanisms are not understood. It has been suggested that retinoic acid synthesis is a closely regulated step of retinol metabolism. In *in vivo* studies, increased plasma retinoic acid concentrations are associated with a reduction in circulating levels of retinol. Using ferret liver, the mechanism of regulation by retinoic acid on hepatic retinol metabolism was studied *in vitro* demonstrating that retinoic acid exerts feedback inhibition of retinal oxidation while stimulating synthesis of retinyl esters in ferret liver.

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GIL-J296 Johnson LD, Ausman LM, Sehgal PK, King NW. A prospective study of the epidemiology of colitis and colon cancer in cotton-top tamarins (Saquinus oedipus). Gastroenterology 1996;110:102-15.

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GENETICS LABORATORY

CRIS: Regulation of Gene Expression in Nutrient Metabolism

MISSION

To examine the molecular mechanisms by which diet and development regulate metabolic pathways at the genetic level. The major focus is the absorption, storage and utilization of nutrient energy. This process constitutes a complex homeostatic system in mammals, balancing energy intake and expenditure while maintaining energy stores. Consequently, the nutrient regulation of gene expression is highly complex, involving numerous positive and negative stimuli. Studies use both *in vitro* and *in vivo* molecular techniques to determine how individual nutrients activate and suppress transcription. Little is known about the genetic control of the process of lipogenesis which underlies the accumulation of body fat and synthesis of circulating fats. Molecular techniques are now available to study the dietary and hormonal control of lipogenesis in the organ which is most metabolically active, the liver. The current focus is on determining the DNA sequence elements that regulate lipogenic gene transcription in response to diet and hormones; identifying the critical transacting factors that interact with these elements and how they transduce dietary and hormonal signals; and determining how altered transcription of lipogenic genes in diabetes and obesity affect lipogenesis and the response to nutritional stimuli.

INVESTIGATORS

K. Eric Paulson, Ph.D. Laboratory Chief/Scientist II Assistant Professor, Biochemistry and Physiology	Provides leadership to the laboratory. Involved in *in vitro* molecular studies and productionof transgenic mice for *in vivo* studies of lipogenic and gene expression.
Helen Palmer, Ph.D. Scientist II	Investigates aging-related changes in signal transduction and gene expression in the mammalian liver. Specific focus is on alterations in membrane lipid/vitamin E composition with aging and how these changes affect MAP kinase cascades and AP-1 activation of genes.

TECHNICAL SUPPORT

Creighton Tuzon, B.A., Research Technician
Waleed Niazy Hassan, M.D., Graduate Research Assistant
Kim G. Mendelson, B.S., Graduate Research Assistant
Liang-Ru Wen, M.S., Graduate Research Assistant
Wang Zheng, B.S., Graduate Research Assistant

CURRENT PROJECTS

- Identification of DNA sequence elements regulating glucose-6 phosphate dehydrogenase gene expression by thyroid hormone.

- Nutritional evaluation of lipogenic mutant mice (MOD-1, ob, db).

- Definition of positional regulation of lipogenic gene expression in the liver in response to nutritional stimuli.

- Analysis of the role of the Jun/Fos proto-oncogene family in the regulation of gene expression by oxidants and antioxidants.

- Analysis of the E2F transcription factor family during intestinal differentiation and the establishment and maintenance of gut absorptive and enzymatic activities.

RECENT RESEARCH ACCOMPLISHMENTS

Binding of the Ah receptor to the glutathione S-transferase xenobiotic response element (XRE) is facilitated by C/EBP binding. The glutathione S-transferase XRE has both constitutive and xenobiotic-inducible activity. Findings demonstrate that the XRE is regulated by both the constitutive C/EBP transcription factor and the ligand-activated Ah receptor. In functional testing of the C/EBP-XRE interaction, cotransfected C/EBPa and b factors activated an XRE test promoter in the non-xenobiotic responsive HeLa cell line. Unexpectedly, cotransfected C/EBPa and b factors did not significantly activate the XRE test promoter in the xenobiotic responsive, tissue-specific HepG2 cell line. Mutational analysis of the XRE revealed that the constitutive factor (C/EBP) shares a nearly identical overlapping binding site with the Ah receptor. Finally, Ah receptor binding was enhanced in the presence of C/EBP. These results suggest that both C/EBP and dioxin receptor recognize the same DNA sequence element and that transcriptional regulation could occur by a synergistic interaction between these two transcription factors.

The glutathione S-transferase Ya gene is transcriptionally and positionally regulated in transgenic mice. Transgenic mice have been constructed carrying chimeric DNA constructs with -1.55 kbp of upstream sequence linked to the herpes TK gene. These constructs are transcriptionally inducible as well as pericentrally regulated. Therefore, because these constructs are regulated exactly as the endogenous gene, the positional regulation is controlled at the transcriptional level.

The lipogenic genes are expressed in periportal hepatocytes in response to carbohydrates. The mRNA localization and transcription of three genes coding for enzymes involved in fatty acid synthesis, malic enzyme (ME), glucose 6-phosphate dehydrogenase (G6PDH) as well as fatty acid synthetase (FAS) has been examined. Lipogenic gene expression has been found to be stimulated by carbohydrates within 12 hours in periportal hepatocytes and then broadens to all hepatocytes from 24 to 48 hours. At later times lipogenic gene expression is again restricted to periportal hepatocytes.

A null mutation of the malic enzyme gene results in altered lipogenic gene expression. The mRNA localization and transcription of the lipogenic genes G6PDH and FAS in animals carrying a null mutation in the malic enzyme gene have been examined. Unexpectedly, these animals show both a delay in the transcriptional induction of G6PDH and FAS in the liver by carbohydrates as well as a reversal of the normal positional regulation of gene expression. Results indicate that the induction of the G6PDH and FAS genes by carbohydrates may require specific metabolites which are unavailable in malic enzyme null mutants.

Oxidative damage in the liver results differential induction of the proto-oncogenes c-jun and c-fos. The mRNA localization and transcription of the proto-oncogenes c-jun, junB and c-fos in response to acute oxidative damage in the liver caused by metabolism of CCl_4 has been investigated. Oxidative damage is restricted to pericentral hepatocytes due to the localization of the P450 enzymes to this region. c-Jun transcription is localized to the immediate pericentral necrotic zone and its expression is concomitant with the induction of its target gene, the antioxidative enzyme glutathione S-transferase. Conversely, c-fos and junB are induced in the periportal region, concomitant with the induction of the regenerative response and hepatocyte proliferation. These findings suggest that c-jun and c-fos are regulated by different signal transduction mechanisms which lead to differing cellular responses to oxidative stress.

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LABORATORY FOR NUTRITION AND VISION RESEARCH

CRIS: Effect of Nutrition and Aging on Eye Lens Protein and Protease Function

MISSION

To determine the primary causes of eye lens cataract and degeneration of the macula and to apply that knowledge to extend the useful life of these organs. Current approaches involve defining adequate levels of nutrients during various stages of life which will result in delayed accumulation of damaged proteins in lens and retina, as well as delayed lens opacification and age-related maculopathy. The laboratory pursues this mission principally using clinical/ epidemiological studies and laboratory tests, employing human and other mammalian lens tissue, a variety of animal models, whole lenses in culture, and lens epithelial cells in culture. Since the lens is primarily composed of protein, a significant effort is being made to understand interrelationships between aging, regulation of lens protein metabolism, protease function and expression, and nutrition.

INVESTIGATORS

Allen Taylor, Ph.D. Laboratory Chief/Senior Scientist Professor, Nutrition	Coordinates and designs laboratory animal and clinical projects.
Martin Obin, Ph.D. Scientist II	Studies effects of oxidation, aging, and diet on retinal protein turnover and signal transduction.
Fu Shang, Ph.D. Scientist II	Studies effects of oxidation and aging on protein turnover.

Xin Gong, Ph.D. Research Associate	Analyzes tissues from (calorie restricted, oxidant-challenged and nutrient modified) animal and human studies.

VISITING SCIENTISTS

Patricia Khu, M.D.	Performs ophthalmology examinations.
Yuhui Ying, M.S.	Studies biologic oxidations in relation to suspectibility to ubiquitin- and nonubiquitin-dependent proteolysis.

TECHNICAL SUPPORT

Thomas Nowell, B.S., Research Technician
Mona Scrofano, B.A., Graduate Research Assistant
Esther Epstein, Staff Assistant

CURRENT PROJECTS

- An epidemiological/clinical and nutritional biochemistry examination of the role of nutrition in the occurrence of senile lens opacities in 50-75 year old adults.
- The relationships between nutrition, aging, and function of proteolytic or antioxidative pathways.
- Effect of ascorbate and carotenoid supplementation on ascorbate levels in human serum and eye tissues.
- Determination of the optimal levels of dietary ascorbate for maximal protection of the lens against cataract and cataract-like changes in animal lenses *in vivo* and in culture.
- Effect of dietary restriction and/or dietary antioxidant (glutathione or tocopherol) supplementation on: life extension; health maintenance; and on the onset of senile-type cataract in the Emory and CFW mouse.
- Biochemical and molecular genetic investigations of the beneficial effects of dietary restriction.
- Monitoring DNA integrity and oxidation in animals fed calorically restricted diets.
- Molecular biological investigations regarding function of amino-peptidases.
- Effect of ascorbate supplementation on oxygen or light-induced damage to proteases, ubiquitin-conjugating enzymes, and antioxidant enzymes in cultured whole lenses and cultured lens epithelial cells.

- Response of endogenous protein breakdown to cellular stresses, such as serum starvation, aging, and ultraviolet light or peroxide exposure.

RECENT RESEARCH ACCOMPLISHMENTS

Prophylactic effect of ascorbate against cataract formation. The first two stages of clinical/epidemiological studies regarding correlations between nutrient intake and age-related degeneration of the macula and cataract suggest a prophylactic effect of ascorbate against cataract formation. Prevalence of age-related maculopathy in the Nurses Study appears to be inversely related to with ascorbate status.

Aging compromises function of the lens-protein-editing capability. Quantitative correlations between age, protein damage, and accumulation of ubiquitin conjugates have been established. ALP and ubiquitin-dependent processes for degradation of lens proteins have been identified. Marking of proteins by attachment of ubiquitin (a 76-amino acid polypeptide) is required to initiate proteolysis. Oxidation of proteins makes them more susceptible to removal in part by ubiquitin-dependent pathways. Glycation has variable effects on susceptibility to degradation of protein substrates. It is probable that enhanced antioxidant levels result in less bulk protein damage and prolonged protease function.

Caloric restriction, cataract and cancer. Restricted caloric intake is associated with delayed incidence of cataract, decreased incidence of a variety of cancers, decreased levels of glycohemoglobin, plasma glucose, and ascorbate. The effect of calorie restriction on each of the lens crystallins during aging and cataractogenesis suggests that attenuation of the protein-catabolizing machinery may be causally related to the accumulation of damaged proteins associated with cataract. Dietary antioxidants may offer protection against these insults.

Identification of the structure of leucine aminopeptidase. The structure of leucine aminopeptidase has been solved to 2.3 Å and searches for function are in progress of these enzymes. Kinetic parameters regarding hydrolysis of peptides have been defined, and an affinity label has been synthesized. These are all firsts for the whole class of enzymes, the aminopeptidases. A full-length clone of leucine aminopeptidase has been isolated and tissue-specific expression of this enzyme has been studied. In addition to its use in structural and biochemical studies, the use of this clone, or selected cDNAs, allowed investigators to monitor alteration in expression of this peptidase in lens cells deprived of nutrients/hormones.

SELECTED RECENT PUBLICATIONS

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Huang LL, Jahngen-Hodge J, Taylor A. Bovine lens epithelial cells have a ubiquitin-dependent proteolysis system. Biochim Biophys Acta 1993;1175:181-7.

Wallner BP, Hession C, Tizard R, Frey AZ, Zuliani A, Taylor A. Isolation of bovine kidney leucine aminopeptidase c DNA: comparison with the lens enzyme and tissue-specific expression of two mRNAs. Biochemistry 1993;32:9296-9301.

Taylor A, Peltier CZ, Jahngen EGE Jr, Laxman E, Torre FJ. Use of azidobestatin as a photoaffinity label to identify the active site peptide of leucine aminopeptidase. Biochemistry 1992;31:4141-50.

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LIPID METABOLISM LABORATORY

CRIS: Nutrition, Lipoproteins and Aging

MISSION

To define the interrelationships between lipoprotein metabolism, nutrition and the aging process. To develop recommendations for older adults regarding dietary fat and cholesterol in an effort to minimize cardiovascular risk factors and atherosclerosis. Research focuses on defining the biochemical parameters which identify individuals at risk for premature coronary artery disease and optimal diets which minimize such plasma lipoprotein abnormalities in the elderly; the short and long term regulation of plasma lipoproteins by diet; the nutritional regulation of lipoprotein synthesis and apolipoprotein gene expression *in vitro* and *in vivo*; the nutritional requirements for essential fatty acids with aging; and prevention of diet induced atherosclerosis. Methodologies established in the laboratory include lipoprotein isolation by ultracentrifugation, automated standardized enzymatic lipid analysis, gradient gel electrophoretic analysis of plasma lipoproteins, apolipoprotein isoelectric focusing, apolipoprotein quantitation by enzyme linked immunoassays, stable isotope kinetic studies, fatty acid analysis by gas liquid chromatography, cell culture studies, DNA isolation and genomic blotting analysis, specific mRNA quantitation, DNA amplification and gene cloning and sequencing.

INVESTIGATORS

Ernst J. Schaefer, M.D. Laboratory Chief/Senior Scientist Professor, Medicine and Nutrition	Provides leadership to the laboratory. Directs lipid and apolipoprotein analyses. Conducts nutritional, clinical and population studies.
Alice H. Lichtenstein, D.Sc. Scientist I Associate Professor, Nutrition	Conducts human studies on nutritional regulation of lipoprotein metabolism and apolipoprotein synthesis *in vivo* using stable isotopes. Examines nutritional response of plasma lipoproteins.
Jose M. Ordovas, Ph.D. Scientist I Associate Professor, Nutrition	Examines apolipoprotein synthesis by *in vitro* and *in vivo* studies. Conducts gene analysis and apolipoprotein immunoassays. Examines nutritional regulationof apolipoprotein and lipoprotein receptor gene expression and studies preventionof diet-induced atherosclerosis in animals.
Stefania Lamon-Fava, M.D., Ph.D. Scientist II	Analyzes results of population studies which examine the nutritional, hormonal, and genetic regulation of plasma lipoproteins. Examines nutritional and hormonal control of apolipoprotein A-I gene expression.
Leo Seman, M.D., Ph.D. Research Associate	Investigates the nutritional and genetic regulation of lipoprotein(a).

Francine Welty, M.D., Ph.D.
Research Associate

Examines factors regulating the genetic control of plasma low density lipoprotein response, especially apoB gene mutations to dietary modification.

VISITING SCIENTISTS

Maysa Cendoroglo, M.D.
Montserrat Vilella-Bach, B.S.

TECHNICAL SUPPORT

Judith McNamara, M.T., Senior Research Assistant
Carl DeLuca, B.S., Research Technician
Tatyiana Massov, Research Technician
Doreen Osgood-McWeeney, M.S., Senior Research Technician
Wanda Carrasco, M.S., Graduate Research Assistant
Sahar El Swefy, B.S., Graduate Research Assistant
Jennifer Jenner, M.S., Graduate Research Assistant
Elaine Larsen, M.S., Graduate Research Assistant
Zhengling Li, M.S., Graduate Research Assistant
Zhiyong Sun, M.S., Graduate Research Assistant
Sylvia Borghetti, Medical Secretary

CURRENT PROJECTS

- Effects of diets restricted in total fat, saturated fat, and cholesterol on plasma lipoprotein composition and metabolism using stable isotopes in elderly human subjects.

- Interrelationships between lipoproteins, apolipoproteins, age, gender, genetic lipoprotein disorders, nutritional intake and coronary artery disease in population and metabolic studies.

- Nutritional regulation of lipoprotein composition, metabolism, apolipoprotein and lipoprotein receptor mRNA levels in non-human primates and hamsters.

- Prevention of diet induced atherosclerosis in hamsters.

RECENT RESEARCH ACCOMPLISHMENTS

Determination of the effects of dietary polyunsaturated fat and cholesterol on plasma cholesterol levels. Studies conducted in elderly men and women with normal or moderately elevated blood cholesterol levels indicate that in diets which meet the National Cholesterol Education Program (NCEP) Step-2 criteria (<30% fat, <7% saturated fat, <200 mg cholesterol/day) replacement of corn oil (at 20 percent of calories) with canola oil or olive oil has no significant benefit in low density lipoprotein (LDL) cholesterol lowering. When corn oil was replaced with corn oil stick margarine or beef tallow significant increases in LDL cholesterol were noted. The addition of dietary cholesterol as egg yolk (approximate equivalent of 1 1/2 egg yolks per day) caused significant elevations in LDL cholesterol when added to diets enriched in corn oil or beef tallow.

Examination of varied protein sources on an NCEP diet. Diets meeting NCEP Step-2 criteria when enriched in fish are not superior to diets enriched in poultry in lowering LDL cholesterol. Fish enriched diets were found to suppress the immune response in elderly men and women in contrast to the standard NCEP Step-2 diet.

Investigation of lipoprotein fractions in the Framingham cohort. Examination of the original cohort of the Framingham Heart Study indicate that lipoprotein (a) [Lp(a)] levels are not significantly affected by age or gender and are not related to the levels of other plasma lipoproteins. Approximate 75th percentile values are 25 mg/dl for the total mass of Lp(a).

Establishment of new guidelines for the diagnosis and treatment of lipid disorders. In addition to elevated LDL cholesterol ($\geq$160 mg/dl) other significant risk factors for coronary heart disease have been identified: male over 45 years, female over 55 years not receiving hormonal replacement, hypertension, cigarette smoking, diabetes, high density lipoprotein (HDL) cholesterol < 35 mg/dl, and family history of heart disease in a male parent or sibling prior to age 55, or in a female parent or sibling prior to age 65 years. A risk factor should be subtracted if the HDL cholesterol is > 60 mg/dl. Candidates for diet therapy (NCEP Step 1 or Step 2 diet) include individuals with LDL cholesterol levels $\geq$ 160 mg/dl, LDL cholesterol $\geq$ 130 mg/dl in the presence of two or more heart disease risk factors, and an LDL cholesterol $\geq$ 100 mg/dl in the presence of coronary heart disease.

Investigation of chylomicron production and plasma triglycerides. Dietary studies indicate that after the consumption of a meal rich in fat and vitamin A the intestine produces fatty particles which are metabolized to form chylomicron remnant particles which are then taken up by the liver. This uptake process induces the liver to produce more triglyceride-rich lipoproteins (large very low density lipoproteins). Therefore, about 80 percent of the increase in plasma triglycerides after a fat-rich meal are due to increases in these intestinal particles. The remaining 20 percent is attributed to an increase in the triglyceride content of liver derived lipoprotein particles.

International comparisons of heart disease. Population comparisons between subjects in Framingham, MA and Taipei indicate that Taipei subjects have half the rate of heart disease and twice the rate of stroke compared to Framingham subjects. Taipei subjects consume diets consisting of approximately 9 percent saturated fat, 11 percent monounsaturated fat, 13 percent polyun-saturated fat with approximately 300 mg cholesterol/day. These subjects consume more polyunsaturated fat and less saturated fat than Framingham subjects and have lower LDL cholesterol levels.

Diets high in saturated fat and cholesterol increase LDL cholesterol levels by down regulating LDL receptor activity in non-human primates. About one-third of this effect is due to down regulation of LDL receptor mRNA levels in the liver. Two-thirds appears to relate to changes in membrane fluidity which affect the residence time of the LDL receptor on the cell surface.

As women experience menopause, they develop significant increases in LDL cholesterol levels due to decreases in LDL receptor mediated catabolism. Women receiving estrogen replacement experience a significant reduction in LDL and a significant increase in HDL cholesterol levels. Such alterations are predicted to be beneficial in terms of cardiovascular risk in elderly females.

SELECTED RECENT PUBLICATIONS

LML-J196 Schaefer EJ, Lichtenstein AH, Lamon-Fava S, et al. Effects of National Cholesterol Education Program Step 2 diets relatively high or relatively low in fish-derived fatty acid on plasma lipoproteins in middle-aged and elderly subjects. Am J Clin Nutr 1996;63:24-41.

LML-J296 Contois JH, Mc Namara JR, Lammi-Keefe CJ, Wilson PWF, Schaefer EJ. Reference intervals for plasma apolipoprotein B determined with a commercial immunoturbidomeric assay: results from the Framingham Offspring Study. Clin Chem 1996;42:515-23.

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LML-J496 Cuchel M, Schwab US, Jones PJH, Vegel S, Lammi-Keefe C, Li Z, Ordovas JM, McNamara JR, Schaefer EJ, Lichenstein AH. Impact of hydrogenated fat consumption on endogenous cholesterol synthesis and susceptibility of low-density lipoprotein to oxidation in moderately hypercholesterolemic individuals. Metabolism 1996;45:241-7.

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Schaefer EJ, Lamon-Fava S, Spiegelman D, Dwyer JT, Lichtenstein AH, McNamara JR, et al. Changes in plasma lipoprotein concentrations and composition in response to a low-fat, high-fiber diet are associated with changes in serum estrogen concentration in premenopausal women. Metabolism 1995;44:749-56.

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Brousseau ME, Schaefer EJ, Stucchi AF, Osada J, Vespa DB, Ordovas JM, Nicolosi RJ. Diets enriched in unsaturated fatty acids enhance apolipoprotein A-I catabolism but do not affect either its production or hepatic mRNA abundance in cynomolgus monkeys. Atherosclerosis 1995; 115: 107-19.

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Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PWF, Belanger AJ, O'Leary DH, Wolf PA, Schaefer EJ, Rosenberg IH. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. New Engl J Med 1995;332:286-91.

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Perez-Jimenez F, Espino A, Lopez-Segura F, Blanco J, Ruiz-Gutierrez V, Prada JL, Lopez-Miranda J, Jimenez-Pereperez J, Ordovas JM. Lipoprotein concentrations in normolipidemic males consuming oleic acid-rich diets from two different sources: olive oil and oleic acid-rich sunflower oil. Am J Clin Nutr 1995;62:769-75.

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MINERAL BIOAVAILABILITY LABORATORY

CRIS: Bioavailability of Nutrients in the Elderly

MISSION

To examine the biochemical and physiological basis for changes in absorption and utilization of minerals with aging and to determine the effects of aging on mineral requirements in the elderly. Research is focused specifically on calcium, magnesium and zinc metabolism, and the effects of nutrient and hormonal changes on the expression of genes which modulate mineral metabolism.

INVESTIGATORS

Richard J. Wood, Ph.D. Laboratory Chief/Scientist I Associate Professor, Nutrition	Sets research priorities for the laboratory. Coordinates human studies on mineral and trace element metabolism.
James C. Fleet, Ph.D. Scientist II Assistant Professor, Nutrition	Investigates the molecular mechanism of intestinal mineral absorption and bone formation.
Ohkee Han, Ph.D. Research Associate	Conducts animal studies which investigate the mechanism of intestinal transport and the role of abnormal regulation of iron absorption in genetic iron overload using Caco-2 cells.

TECHNICAL SUPPORT

Jessica Bradley, B.A., Research Technician
Diana Fleming, M.S., Graduate Research Assistant
Andrew Shao, M.S., Graduate Research Assistant

CURRENT PROJECTS

- Assessment of magnesium status and intestinal magnesium absorption using stable isotopes in oteoporotic elderly women.
- Investigation of the relationship between dietary patterns and iron status in a large, free-living adult population.
- Measurement of zinc bioavailability in humans from individual foods and complex meals.
- Evaluation of the importance of end-organ resistance to 1,25-dihydroxyvitamin D action on calcium

metabolism in the aged.

- Effects of growth hormone and insulin-like growth factor (IGF-I) action on intestinal mineral absorption and skeletal metabolism in aged female rats.

- Immunohistochemical studies on age- and hormone-induced changes in the abundance and distribution of vitamin D receptors and calbindin D in rat intestine.

RECENT RESEARCH ACCOMPLISHMENTS

Zinc bioavailability in humans can differ markedly between individual foods. The bioavailability of essential minerals, such as zinc, from the diet is an important determinant of mineral status. A novel absorption technique in humans has been used to demonstrate that zinc from beef is three times more bioavailable than zinc derived from fortified breakfast cereal. In order to better define the scientific basis of the Recommended Dietary Allowances for minerals, projects are currently underway to utilize this new research tool to investigate the important dietary determinants of mineral bioavailability from human diets.

Hypochlorhydria does not lead to an impairment of mineral absorption. It has been generally believed that the reduced stomach acid secretion, a frequent condition among the elderly, may result in impaired mineral absorption and declines in mineral status. Recent studies have demonstrated that drug-induced hypochlorhydria in an animal model did not result in impaired zinc bioavailability. In addition, findings from human studies indicated that age-associated hypochlorhydria may have less of an impact on mineral status in the elderly than previously believed.

Development of a novel dual isotope magnesium absorption test. This test uses 25 mg and 26 mg, two non-radioactive magnesium isotopes. Impaired magnesium absorption and status may be an important risk factor for age-related disorders of the immune, cardiac, and skeletomuscular systems. Fractional magnesium absorption has been studied in normal young and elderly women using this new technique and shown to be equivalent.

Growth hormone enhances the anabolic effect of parathyroid hormone (PTH) on bone in the aged animal. PTH is one of the few hormones to positively affect bone remodeling in osteoporotic humans. Recent studies indicate that growth hormone treatment is needed to achieve a maximal anabolic effect of PTH in the aged skeleton. This finding may be related to the corresponding elevation of calcium and phosphorus absorption seen with this combined hormonal treatment. The mechanism of these effects seen in the intestine involve a vitamin D-independent induction of calbindin D, a putative calcium transport protein. Results indicate that more complex treatment approaches may be needed to optimize bone formation in the aged skeleton.

Caco-2 cells are an unique *in vitro* cell culture system to study various aspects of mineral metabolism in the human enterocyte. Caco-2 cells are an intestinal cell line derived from a human colon carcinoma. These cells spontaneously differentiate in culture into small intestine-like cells. Caco-2 cells possess a vitamin D receptor, increase calbindin D mRNA in response to 1,25-dihydroxyvitamin D, and transport calcium in a vitamin D-dependent manner via a lysosomal pathway. These cells represent an important experimental model to investigate the mechanism of vitamin D-dependent calcium transport. Laboratory studies have also shown that Caco-2 cells display vitamin D-dependent zinc transport.

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NUTRITIONAL IMMUNOLOGY LABORATORY
and
Vascular Biology Program

CRIS: Nutrition, Aging, and Immune Response

To investigate the role of dietary components, in particular antioxidants, prooxidants and lipids, and their interactions with other environmental factors in age-associated changes of the immune and inflammatory responses and vascular function. To reverse and/or delay the onset of immunological and age-related changes by appropriate dietary modifications. To use the immune response and oxidative stress as biologically meaningful indices in determining dietary requirements. To understand the molecular mechanism(s) of antioxidants and prooxidants in the modulation of immune and inflammatory cells and the cellular components of the vascular system during aging and in the development of age-associated disease. Cell culture systems, animal models, and human volunteers are used to explore changes in immune response and vascular function as affected by nutrients using cell-cell interactions, biochemical indices, and molecular markers of oxidative stress at the cellular and whole organism levels to determine specific dietary requirements.

INVESTIGATORS

Simin Nikbin Meydani, D.V.M., Ph.D. Laboratory Chief/Senior Scientist Professor, Nutrition	Provides overall direction to the laboratory. Develops research programs. Designs experimental protocols.
Mohsen Meydani, D.V.M., Ph.D. Senior Scientist, Vascular Biology Program Associate Professor, Nutrition	Develops research programs. Designs experimental protocols.
Alison Beharka, Ph.D. Scientist III, Nutritional Immunology Laboratory	Studies the mechanism of vitamin E-induced enhancement of immune response and the effect of vitamin E on viral and bacterial infection.
Dayong Wu, M.D. Scientist III, Nutritional Immunology Laboratory	Studies the effect of dietary lipids on the immune response of older subjects and the effects of vitamin E on eicosanoid metabolism.
Keith Martin, Ph.D. Research Associate, Vascular Biology Program	Studies the effect of antioxidants and fatty acids on immune cell/endothelial cell interactions.

TECHNICAL SUPPORT

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CURRENT PROJECTS

- Definition of vitamin E requirements for optimal immune responsiveness and platelet aggregation in healthy young and older adults.
- Elucidation of the mechanism of the immunostimulatory effect of vitamin E in aged mice.
- Elucidation of molecular mechanisms of age-associated increases in PGE_2 production.
- Elucidation of the mechanisms of the vitamin E-induced increase in resistance to influenza infection.
- Effect of black currant seed oil on the immune response and oxidative stress of older subjects.
- Mechanisms of ß-carotene-induced enhancement of natural killer cell activity.
- Elucidation of mechanisms of (n-3) PUFA-induced suppression of cell-mediated immunity.
- Effect of *trans* fatty acids on immune response of humans.
- Effect of antioxidants on immune response, longevity, and resistance to infectious diseases.
- Effect of voluntary dietary energy restriction on the immune response of the elderly.
- Antioxidants, fatty acids, and mechanisms of endothelial cell/monocyte adhesion.
- Vitamin E and fish oil interactions on LDL oxidation.
- Vitamin E requirements of elderly consuming fish-derived (n-3) PUFA.
- Effect of antioxidants on longevity, oxidative stress and mitochondrial DNA deletion in mice.
- Effect of dietary restriction on the development of atherosclerosis in hamsters.
- Effect of aging, homocysteine, glutathione, and its precursors on monocyte and macrophage function as related to the pathogenesis of atherosclerosis.

RECENT RESEARCH ACCOMPLISHMENTS

Long-term supplementation with vitamin E enhances immune response in young and older adults. Preliminary data analysis from a double-

blind, placebo-controlled study indicates that long term (6 months) supplementation with vitamin E significantly improves the delayed-type hypersensitivity skin response in young and older adults. The improvement is more dramatic in older than young adults (37 vs 97%, respectively). Furthermore, data indicate that long-term supplementation with different doses of vitamin E enhances *in vitro* and *in vivo* indices of the immune response in healthy elderly. Supplementation with 200 and 800 IU/day causes a significant improvement in several indices of T cell-mediated function while 60 IU/day results in a marginally significant improvement in some indices of the immune response.

Short-term supplementation with large doses of vitamin E does not have an adverse effect in healthy elderly. Safety of 30 day supplementation with 800 IU/day of tocopherol was assessed in healthy elderly. No adverse effect was noted in weight gain, hepatic and renal function indices, blood glucose, protein, lipids, tyroid hormones, hematological status, and the status of other nutrients.

In vitro GSH supplementation increases mitogenic response and IL-2 production by human PBMC. In vitro supplementation with GSH (2-10 mm) increased mitogenic response, and IL-2 production and decreased PGE_2 and LTB_4 production by PBMC from both young and older subjects.The percent increase was significantly higher in older subjects.

Effect of age on arachidonic acid metabolism: implication for T cell proliferation. Splenocytes from old mice synthesize significantly more PGE_2 [cyclooxygenase (COX) product] and LTB_4 and LTC_4 (5-lipoxygenase (5-LO) product] than young mice. No age-related difference in production of 12-HETE or 15-HETE or in H_2O_2 production was observed. Inhibition of COX and not of 5-LO or H_2O_2 production significantly improved mitogenic response of old mice. It is concluded that of the AA metabolites, PGE_2 is the major contributing factor to the age-associated decrease in T cell proliferation.

Fish derived (n-3) PUFA enriched diets have profound effects on the immune response. Long-term (6 months) feeding of low-fat, low-cholesterol, (n-3) PUFA-enriched diets decreased production of IL-6, TNF, granulocyte-monocyte colony stimulating factor, lymphocyte proliferation and the delayed hypersensitivity skin reaction. These effects were not observed when essentially the same diet [but low in fish-derived (n-3) PUFA] was fed for the same amount of time.

Eicosanoids derived from arachidonic (AA) and eicosapentanoic acids (EPA) inhibit T cell proliferation. To determine the mechanism of fish oil-induced decrease in lymphocyte proliferation, the effect of AA- and EPA-derived eicosanoids on mitogenic response were compared in mice. EPA-derived eicosanoids (PGE_3 and LTB_5) were found to be more effective than AA-derived eicosanoids (PGE_2 and LTB_4) in inhibiting lymphocyte proliferation. This is a novel finding in that in most other biological functions e.g. platelet aggregation the EPA-derived eicosanoids are less effective than AA-derived eicosanoids.

The suppressive effect of fish oil on lymphocyte proliferation is due to increase production of (n-3) PUFA-derived eicosanoids a s well as compromised tocopherol status. Mice were fed diets containing 10% by weight of fish oil, or a mixture of oils representing an average American diet. Fish oil-fed mice had significantly lower mitogenic response to T cell mitogens. This effect was reversed by in vitro addition of indomethacin (cyclooxygenase inhibitor) and vitamin E. In another study, primates fed 3.5% marine-derived (n-3) PUFA with an adequate level of tocopherol showed higher lymphocyte proliferation and IL-2 production than those fed an oil blend based on the average American diet. These findings together with the observation that EPA-derived eicosanoids are more suppressive than AA-derived eicosanoids indicates that increase in production of both enzymatic and non-enzymatic lipid peroxidation contribute to the suppressive effect of fish oil.

ß-Carotene supplementation enhances natural killer (NK) cell activity in older subjects. Natural killer cell activity was assessed in a sub group of subjects participating in a Harvard Physician's Health Trial who had consumed a placebo or ß-carotene supplement (50 mg every other day) for 12 years. Older subjects had lower NK activity than young subjects. Supplementation with ß-carotene significantly increased NK activity in older subjects. This effect was not due to a decrease in percent NK cells.

Progressive resistance training does not affect T cell-mediated function in young or old subjects. Young and old healthy subjects and middle-aged subjects with rheumatoid arthritis who underwent twelve weeks of progressive resistance training did not show any benefit or adverse effect in in vivo and in vitro indices of T cell-mediated function.

Vitamin E modulation of oxidative stress-induced changes in endothelial cells and monocyte adhesion. Supplementing human endothelial cells with vitamin E decreased production of reactive oxygen species by endothelial cells and increased resistance of cell oxidative injury from hypoxia/reoxygenation, particularly in cells from the saphenous vein which is often used in grafting procedures in heart bypass surgery. Supplementing human aortic endothelial cells with vitamin E to the level achieved with oral supplementation prevented endothelial cell injury from oxidative stress induced by AAPH, a stable free radical generator, and oxidized LDL and

modulated IL-1 and PGI_2 production by endothelial cells. Human aortic endothelial cells exposed to increased doses of LDL showed increased adhesion to monocytes. Vitamin E supplementation of endothelial cells decreased the expression of adhesion molecule ICAM-1 and reduced adhesion of monocytes to endothelial cells. Exposure of endothelial cells to oxidant and native LDL activated NF-κB nuclear transcription factor involved in oxidative stress-induced signal transduction.

Carotenoids and tocopherol concentration in plasma, peripheral blood mononuclear cells, and red blood cells following long-term ß-carotene supplementation. Plasma tocopherol status, red blood cells, and peripheral blood mononuclear cells were investigated in blood samples of men participating in the Harvard Physicians' Health Trial who consumed either a placebo or a β-carotene supplement (50 mg every other day) for 12 years. Long-term β-carotene supplementation had no significant effect on the concentration of other carotenoids or tocopherols in plasma or blood cells.

Fish oil, vitamin E and probucol in renal segmental sclerosis. Increases in blood cholesterol and LDL levels not only precipitates development of atherosclerosis, but can cause sclerosis in nephron units. Supplementing high cholesterol diets with probucol, a synthetic antioxidant, fish oil, vitamin E, or a combination of these, for 4 weeks reduced the progression of inflammatory kidney injury in rats as indicated by a decrease in glomerular macrophage population and reduction in occurrence of glomerular sclerosis in the kidney remnant model of progressive kidney disease.

Acute phase response in exercise: Association between vitamin E, cytokines, lipid peroxidation, and muscle proteolysis. In a double-blind, placebo-controlled protocol, male subjects were supplemented with 800 IU/day vitamin E for 2 months. Significant increases in *ex-vivo* IL-1β and TNF-α production were observed following eccentric exercise in the placebo group. The increase in IL-1β production was not observed in the vitamin E-supplemented group. Il-6 production was not increased by exercise. Urinary 3-methylhistidine excretion correlated with mononuclear cell secretion of IL-1β and PGE_2 indicating that these mononuclear products contribute to regulation of muscle proteolysis. Following eccentric exercise, all vitamin E-supplemented subjects excreted less urinary lipid peroxides than the placebo group. Vitamin E levels in exercised muscle decreased, and muscle lipid-conjugated dienes increased in placebo subjects. These indices indicated that vitamin E protects against exercise-induced oxidative injury.

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NUTRITION AND EXERCISE PHYSIOLOGY LABORATORY

CRIS: Relationship Between Aging, Functional Capacity, Body Composition and Substrate Metabolism and Needs

MISSION

To explore the interaction between nutrition, exercise and aging. To understand how physical activity affects nutrient requirements and functional capacity in the elderly. The extent to which aging alters the adaptive responses to increased physical activity is largely unknown, particularly its effects on protein metabolism. The laboratory is focusing its activities on the metabolism and requirements of several macronutrients and how they change with age and activity. Stable isotope probes and the euglycemic glucose clamp technique are used to establish how energy expenditure, body composition and the turnover of whole body nitro-gen and glucose vary in the population with increasing age, particularly with regard to changes in amount of physical activity. Through the use of these techniques, it can be established how these changes affect substrate requirements.

INVESTIGATORS

Maria A. Fiatarone, M.D. Laboratory Chief/Scientist I	Examines the effects of exercise and nutrient status on functional capacity of the oldest old.
Miriam E. Nelson, Ph.D. Associate Laboratory Chief/Scientist II Assistant Professor, Nutrition	Conducts research on the effect of exercise and diet on bone health and body composition in the elderly.
Carmen Castaneda, M.D., Ph.D. Scientist III	Investigates the interaction of exercise, dietary protein, and protein requirements in the elderly.
Roger A. Fielding, Ph.D. Scientist III	Investigates the effects of concentric and eccentic muscle actions on changes in protein turnover.
Wenjing Ding, M.D., M.Ed. Research Associate	Studies the effects of age, nutrition and exercise on skeletal muscle, histochemistry and ultrastructure.
Virginia Hughes, M.S. Research Associate	Studies the effects of a high carbohydrate, high fiber diet on glucose metabolism in subjects with impaired glucose tolerance.

VISITING SCIENTISTS

Michele Porter, Ph.D.
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Studies the impact of progressive resistance training on body composition and energy expenditure in physical activity in elderly women with chronic heart failure.

TECHNICAL SUPPORT

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CURRENT PROJECTS

- Effects of resistance training on body composition in postmenopausal women.
- Weight reduction and resistance training in postmenopausal women.
- Resistance training and nutritional supplementation in frail elders.
- Effects of exercise training on functional status in home-bound elders.
- Functional implications of weight loss in the very old.
- Chronic adaptation to endurance and resistance training in functionally dependent older women.
- Exercise-induced muscle proteolysis and ubiquitin-mediated skeletal muscle protein degradation.
- Function, morphology and expression of the insulin responsive glucose transporter protein in aging skeletal muscle.

RECENT RESEARCH ACCOMPLISHMENTS

Muscle damage is related to the accumulation of cellular mediators of inflammation. Needle biopsies of the vastus lateralis were performed prior to, immediately after, and 5 days after 45 minutes of downhill running in nine untrained men. Significant increases in muscle interleukin-1 (IL-1) and neutrophils was seen. Neutrophil accumulation was correlated to intracellular Z-band damage as well as IL-1, indicating an association between myofibrillar disruption and cellular mediators of inflammation.

Functional capacity is highly related to leg power in the very old. The leg extensor power was measured in very old residents of a chronic care hospital. Performance measures were obtained by measuring the time to rise from a chair, climb stairs, and walk. Leg extensor power was significantly correlated with each of the performance measures used. Threshold values of 1.2 watts per kilogram of body mass from both legs appear to be needed for unassisted walking. Those who required a walker had less than half of the leg power of those who could walk freely, and over 86 percent of the variability in walking speed in women was explained by leg power, suggesting that poor performance in walking was due to poor muscle function rather than poor balance.

A high carbohydrate, high fiber diet combined with aerobic exercise does not improve glucose tolerance. Glucose intolerant men and women consumed a high carbohydrate, high fiber diet while maintaining body weight for 12 weeks. One group remained sedentary while the other group exercised at 70 percent of maximal heart rate reserve 4 days per week. There was no improvement in glucose tolerance or insulin-stimulated glucose uptake. In addition, HDL cholesterol levels declined while plasma triglyceride levels increased. These data indicated that without weight loss a high carbohydrate diet, even when combined with exercise, has little benefit in men and women at high risk for developing diabetes.

Exercise-induced muscle damage has a long lasting effect on whole-body protein metabolism. Using a primed constant infusion of 1-[^{13}C] leucine, no difference in flux between old and young men was observed. However, protein turnover was greater in the older men when expressed as a function of fat-free and muscle mass, reflecting a redistribution of whole body protein metabolism with higher protein turnover of the non-muscle compartment.

Resistance training and nutritional supplementation in frail elders. In the frail elderly, loss of function may be related to undernutrition and extreme sedentariness. In a randomized, controlled factorial design, this project examined the effects of 10 weeks of resistance training and complete nutritional supplementation (360 kcal/day). Parameters examined included ultrastructural analysis, muscle fiber size, force production, regional muscle mass, mobility, and functional independence. Resistance training resulted in significant improvements on muscle strength, gait speed, stair climbing ability, and overall activity levels. The nutritional supplement had no independent or interactive effects on these outcomes, and suppressed habitual dietary intake in non-exercising subjects. These results suggest that physical frailty among the very old is partially due to disease and is correctable by exercise.

Adaptation to dietary protein intakes in the elderly. The adequacy of the current RDA for protein in the elderly and the long-term (8 weeks) mechanisms of adaptation or accommodation to two different dietary protein intakes, 0.45 and 0.92 g protein/kg/day, were tested in older women. The 0.45 diet resulted in a significant lowering of fat-free mass as assessed by whole body potassium accompanied by a significant decrease in cellular immune response, muscle strength, power and neuromuscular muscle function compatible with accommodation. Subjects fed 0.92 g/kg/protein per day successfully adapted to the diet.

Weight bearing exercise and calcium increase bone density in post-menopausal women. A 1-year program of walking 4 days per week and/or calcium supplementation in women of average age of 60 years produced a significant improvement in spinal bone density due to exercise and a significant increase in femoral neck density due to calcium. Exercise also resulted in a 7 percent increase in aerobic capacity after one year, as compared to a 7 percent decrease in the sedentary women.

Effects of dietary protein intake and resistance training on protein and energy metabolism and body composition. Twelve men and women aged 56-80 years underwent 12 weeks of whole body resistance training while consuming either 0.8 or 1.6 g protein/kg/day. Energy needs for weight maintenance were increased by 15 percent with resistance training. Nitrogen balance was estimated to require 1.0 g protein/kg/d, considerably greater than current recommendations for healthy adults. Nitrogen retention increased during resistance training at both levels of dietary intake. The level of protein intake did not influence the physiologic response to resistance training in terms of muscle strength or body composition.

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VITAMIN K LABORATORY

CRIS: Function and Metabolism of Vitamin K and Vitamin K-dependent Proteins During Aging

MISSION

To determine the contribution that various forms of vitamin K and other fat soluble vitamins make to health and well-being during aging. Vitamin K is responsible for introducing unique calcium-binding sites (gamma-carboxyglutamic acid residues) into vitamin K-dependent proteins. Prior to 1976 the only known proteins (prothrombin, factors VII, IX, & X) thought to be vitamin K-dependent were involved in blood coagulation. Today we know that there are other vitamin K-dependent proteins (Proteins C and S) involved as anticoagulants. Three others (osteocalcin, matrix gla protein & protein S) are involved in bone biology. Matrix protein, originally discovered in cartilage, has been shown to be synthesized in other tissues in the body in larger quantities than in bone. Gas6, a new vitamin K-dependent protein, shows some homology to protein S and serves as a ligand for the activation of membrane bound tyrosine kinases in a variety of tissues. This observation strongly suggests a role for vitamin K in many areas outside of its well-established role in regulating blood clotting. With these new discoveries in mind, our goals are to develop new methods for the biochemical, functional and dietary assessment of vitamin K nutritional status and to determine the nutritional sources, bioavailability and requirements of vitamin K in humans. Epidemiological studies can examine dietary vitamin K intake and its relationship to cardiovascular disease, osteoporosis and bone diseases and various cancers.

INVESTIGATORS

James A. Sadowski, Ph.D. Laboratory Chief/Scientist I Associate Professor, Nutrition	Provides leadership for the laboratory and directs studies on the function and metabolism of vitamin K and other fat-soluble vitamins.

Sarah L. Booth, Ph.D.
Senior Research Associate

Develops vitamin K nutrient databases and dietary intake tools to assess vitamin K nutritional status. Conducts epidemiological and clinical studies on vitamin K in relationship to requirements and disease.

TECHNICAL SUPPORT

Kenneth W. Davidson, M.S., Senior Research Assistant
Maureen O'Brien Morse, B.A., Research Assistant
Kathryn Tsaioun, M.S., Graduate Research Assistant

CURRENT PROJECTS

- Determination of the RDA for vitamin K in an aging human population using controlled metabolic studies.

- Purification, characterization and functional evaluation of vitamin K-dependent proteins (osteocalcin, matrix gla protein and gas6) not involved in blood coagulation and synthesized by extrahepatic tissues.

- Analysis of foods and beverages for the development of a database for the determination of dietary vitamin K intake.

- Determination of the adequacy of the American diet for the current RDA for vitamin K.

- Determining, through epidemiological studies, whether there is a relationship between vitamin K nutritional status and cardiovascular disease, bone disease and cancer.

- Investigating the role of the vitamin K content in the diets of individuals taking the oral anticoagulant warfarin for the prevention of heart attacks and strokes. Warfarin is a direct antagonist of vitamin K action and as a result decreases the ability of the blood to clot.

- Characterizing factors that influence the bioavailability of vitamin K from various food sources and its interaction with the absorption and metabolism of other fat-soluble vitamins and their precursors.

- Determining the biological availability and function of dihydro-vitamin K_1 present in foods prepared from partially hydrogenated vegetable oils. This newly discovered form of vitamin K_1 could make a considerable impact on the vitamin K content of the typical American diet.

- Investigating the role of gas6, a newly discovered vitamin K-dependent protein that is a ligand for membrane tyrosine kinases and may be a regulator of cell growth and differentiation through activation or inactivation of signal transduction pathways.

RECENT RESEARCH ACCOMPLISHMENTS

Undercarboxylated osteocalcin as an indicator of vitamin K nutritional status. An immunochemical assay (radioimmunoassay) has been developed that can differentiate between carboxylated and undercarboxylated osteocalcin. which is produced when dietary vitamin K is too low. The functional significance of this observation has yet to be determined, however, recent studies from other research groups are suggesting that vitamin K nutritional status may be related to the development of various metabolic bone diseases. Undercarboxylated osteocalcin levels were found to be inversely proportional to vitamin K plasma levels and positively correlated with undercarboxylated forms of prothrombin (PIVKA-II).

The vitamin K content of the American diet as determined by analysis of the US Food and Drug Administration Total Diet Study. This study described the analysis of 261 food samples from the US Food and Drug Administration Total Diet Study. Food items which approximate the usual dietary practices of different groups of Americans, were purchased from several supermarkets in various cities several times a year. Green, leafy vegetables appeared to be the predominant dietary source of vitamin K followed by vegetable oils that are derived from vegetables or seeds containing vitamin K (soybean, canola, olive). Some mixed dishes contained moderate amounts of vitamin K that are attributable to the vegetable oils used in their preparation. Other foods, such as certain meats, brewed beverages, soft drinks and alcoholic beverages, contained negligible amounts of vitamin K. These data improve the ability of research scientists and dietary consultants to more easily determine dietary contributions of vitamin K and allow for the adjustments of dietary intake through manipulation of the diet. The results of this study when introduced into a model for food consumption, indicate that the median intakes of vitamin K for most age and gender groups in the United States is at or slightly lower than the RDA for vitamin K.

A provisional table of the phylloquinone content of foods. In response to the need for tabulated data for vitamin K_1, the vitamin K_1 content of foods was evaluated. An effort was made to include vitamin K values representative of food in the retail market. The data indicate that leafy, green vegetables, and certain legumes and vegetable oils are good sources of vitamin K_1. Vitamin K_1 distribution in plants is not uniform, with higher concentrations found in the outer leaves as compared to the pale inner leaves. Fruit and vegetable peels appear to have higher concentrations than the fleshy portions. The limited data on the vitamin K_1 content of foods needs to be expanded to include other commonly-consumed foods, including prepared foods.

Evaluation of an HPLC method for the determination of phylloquinone in food. The amount of phylloquinone in five foods (vegetable juice, whole milk, spinach, ground beef, and a bagel) representative of the five food groups was determined by applying a highly sensitive high performance liquid chromatography (HPLC) method that incorporates postcolumn chemical reduction of the quinone followed by fluorescence detection of the hydroquinone form of the vitamin. The mean phylloquinone content of ten, 100 gram samples of fresh spinach leaf was 299.5μg, 0.3μg for milk, and 4.7 μg for vegetable juice. The content of an individual 100 gram sample of ground beef was 2.57 μg and 0.39 μg for a bagel. This method is currently being used to improve both the quality and quantity of data for the phylloquinone contact of food to enable researchers to make accurate and reliable estimates of the vitamin K content of the typical American diet.

Decreases in urinary gamma-carboxyglutamic acid, plasma vitamin K1 and a rise in plasma PIVK-II have been observed in human subjects with diet-induced vitamin K deficiency. A 15-30 percent decrease in the concentration of urinary gamma-carboxyglutamic acid (gla), a decline in plasma vitamin K_1 concentration below the normal range and a rise in the plasma PIVK-II (abnormal prothrombin antigen) were observed in subjects given a mixed diet low in vitamin K_1 (10 mg/day) for two weeks. The deficiency did not affect blood coagulation as observed by prothrombin time, activated partial thromboplastin time or specific factor (factor VII and protein C) activities. The decline in urinary gla could not be accounted for by the known vitamin K-dependent clotting factors and may reflect changes in other vitamin K-dependent proteins such as bone gla protein or osteocalcin and matrix gla protein.

Diet-induced vitamin K deficiency among older adults suggest age-related change in vitamin K requirements. Elderly subjects were more resistant to developing biochemical signs of vitamin K deficiency as assessed by decreased urinary gla levels even though plasma vitamin K_1 decreased below the normal level and PIVKA-II levels increased in this group. Plasma vitamin K_1 and urinary gla increased in response to supplementary vitamin K_1 (15 to 40 mg/day).

Antibodies for bone and matrix gla protein can be employed to determine the effects of vitamin K depletion. Specific immunochemical assays have been developed for these proteins using polyclonal antibodies produced in rabbits using synthetic peptides that correspond to specific epitopes on each of the vitamin K-dependent proteins. Specific coupling techniques have been developed

to synthesize peptides containing fully carboxylated and partially carboxylated domains in order to develop site specific, carboxyl specific antibodies that will be useful in determining the degree and sequence of carboxylation of these proteins. A site specific antibody for matrix gla protein has been identified that appears to be dependent on the absence of carboxylation at the glutamic acids and may detect non-carboxylated forms of matrix gla protein.

Specific biochemical defects have been demonstrated from vitamin K deficiency in newborn infants. Protein C is a vitamin K-dependent protein that functions as a natural anticoagulant *in vivo*. It has been shown that a considerable percentage of newborn infants synthesize protein C but are not able to efficiently carboxylate the protein. In addition, the levels of vitamin K present in the newborn were found to be extremely low, possibly predisposing them to the observed carboxylation deficiency associated with protein C. The same defect was also observed with prothrombin but was not as severe. In some infants, an accumulation of vitamin K epoxide, a normal metabolite of vitamin K was observed at levels indicating an immaturity in the hepatic enzymes responsible for the cyclic interconversion of vitamin K in the vitamin K cycle. This defect may also be respon-sible for some of the carboxylation defects observed in the newborn infants. These observations have been used as a model for studying vitamin K deficiency in humans.

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VITAMIN METABOLISM LABORATORY

CRIS: Bioavailiability of Nutrients in the Elderly

MISSION

To study the bioavailability of water soluble vitamins in the aging population and determine the effect of aging on vitamin requirements. To examine the basis for the absorption, utilization, and excretion of water soluble vitamins from food in the maturing and elderly population. To assess vitamin status and its relationships to drug intake and chronic diseases. To examine the impact of subclinical vitamin deficiencies on the integrity and function of body physiology.

INVESTIGATORS

Jacob Selhub, Ph.D. Laboratory Chief/Senior Scientist Professor, Nutrition	Provides leadership and guidance to the laboratory. Studies folate and B_{12} metabolism and the pathogenesis of homocysteinemia.
Irwin H. Rosenberg, M.D. Program Director/Senior Scientist Professor, Medicine and Nutrition	Provides overall research direction.
Joel B. Mason, M.D. Scientist I Associate Professor, Medicine	Examines intestinal folate absorption and the relationship between dysplasia and folate deficiency.
Robert Salomon, M.D. Scientist II Assistant Professor, Pathology	Studies the pathophysiology of homocysteinemia using molecular biology approaches.
Pamela Bagley, Ph.D. Research Associate	Genotypes methylene-tetrahydrofolate reductase. Develops food folate bioavailability methodology.
Elizabeth Ross, M.D. Clinical Nutrition Fellow	Conducts cost effectiveness analysis of nutritional interventions in primary care.

VISITING SCIENTISTS

Hyun Wook Baik, Ph.D.
Sang Woon Choi, M.D.

TECHNICAL SUPPORT

Tamara LaVesque, B.S., Clinical Coordinator
Marie Nadeau, M.S., Senior Research Assistant
Farrahraz Esfandiari, M.S., Graduate Research Assistant
Silvina Furlong, M.S., Graduate Research Assistant
Lori Lathrop, R.D., M.S., Graduate Research Assistant
Trudy Hedrick, Staff Assistant

CURRENT PROJECTS

- Food folate bioavailability.
- The use of deuterium labeled folate for studies of folate bioavailability in humans.
- Analysis of folate distribution in tissues.
- Effects of antiepileptic drugs, alcohol, antifol agents, choline, and vitamins B_6 and C deficiencies on folate status.
- Nutritional assessment of vitamin status and the relationship of subclinical vitamin deficiencies to physical and cognitive functions.
- Homocysteinemia pathogenesis and pathophysiology and its identification as a risk factor for occlusive vascular disease.
- The role of B vitamins and sulfur amino acid metabolism in health and disease.
- The relationship between folate deficiency and dysplasia.

RECENT RESEARCH ACCOMPLISHMENTS

Vitamin status and intake as primary determinants of homocysteinemia the elderly. Homocysteine levels of 68-96 year old individuals of the Framingham Heart Study cohort exhibited strong inverse associations with plasma folate levels. Homocysteine demonstrated weaker, inverse associations with plasma vitamin B_{12} and PLP. Similar inverse associations were seen between homocysteine and folate and vitamin B_6 intakes, but not vitamin B_{12}. Prevalence of elevated homocysteine levels was greatest among subjects with low folate status. Inadequate plasma concentrations of one or more B vitamins appear to contribute to two-thirds of the cases of homocysteine elevations. These results indicate a strong association between homocysteine concentration and folate, vitamin B_{12} and vitamin B_6 status. It is possible that a substantial majority of the cases of high homocysteine in this aged population can be attributed to vitamin status.

Elevated plasma homocysteine and carotid artery stenosis. In a cross-sectional survey of the Framingham Heart Study cohort, carotid doppler ultrasound examinations were performed and homocysteine levels were measured in men and women age 67-96 years. Individuals were categorized according to the highest degree of stenosis in either vessel. Age-adjusted homocysteine levels were linearly associated with the degree of carotid stenosis. Elevated homocysteine levels were highly attributable to lower levels of serum folate, vitamins B_6 and B_{12}. However, homocysteine was still related to stenosis after taking serum folate

and vitamins B_6 and B_{12} into account. In multivariate prediction models of carotid stenosis that included age, homocysteine, HDL cholesterol, systolic blood pressure and cigarette smoking, homocysteine remained a significant predictor in men but not in women. These data suggest that homocysteinemia is associated with the degree of stenosis in a major vessel and that inadequate vitamin status may underlie the tendency toward elevated homocysteine levels in the elderly.

B vitamin supplementation lowers homocysteine levels in heart disease. Patients undergoing angioplasty were assessed for levels of homocysteine, folate, vitamin B_{12} and pyridoxal phosphate (PLP). Forty-eight percent of the 197patients screened had an elevated homocysteine level. Low PLP levels were seen in 37 percent of the cases, and low B_{12} in 4 percent. Of those with elevated homocysteine levels, 49 percent had low PLP levels. Relationships between homocysteine and folate or B_{12} did not reach significance. Those with elevated homocysteine and normal vitamin levels were enrolled in a double-blind randomized trial, receiving either supplemental folic acid or a placebo daily for 30 days. Both groups received vitamins B_{12} and B_6 daily for another thirty days. Folic acid alone or B_{12} with B_6 independently lowered homocysteine. The relationship between homocysteine and pyrido-xine in those with vascular disease requires further evaluation. Elevated homocysteine in this population may be treated with supplemental folic acid or with vitamins B_6 and B_{12}.

Combined affinity and ion pair column chromatographies for food folate analysis. Folate exists in forms which differ by oxidation states, the one carbon substitution of the pteridine ring and by the number of glutamate residues. A method developed in this laboratory which combines affinity chromatography with ion pair high performance liquid chromatography (HPLC) provides simultaneous information on the structural nature at both ends of the folate molecule. This study sought to determine if the Affinity/ HPLC method is suitable for analyzing food folates in ten foods. Results showed variability of folate distribution in the various products ranging from a single derivative of 5-methylH4PteGlu found in egg yolk, to a more complex mixture which includes 5-methyl-, 5-formyl-, 10-formyl-, and unsubstituted tetrahydrofolate with 5 glutamate residues found in lima beans. The method appears to be reliable as the measured variability amounted to an average of 10 percent, while total folates obtained by integrating the concentration of individual folates were comparable to total folates estimated using the more traditional microbial assay method.

Folate synthesized by the intestinal microflora assimilated by the human host. Unlike mammalian tissues, certain intestinal microflora are capable of *de novo* synthesis of a number of vitamins including folate. Bacterial synthesis of folate involves the condensation of p-aminobenzoic acid (PABA) with dihydropterin and then with glutamic acid to form dihydrofolate. This study sought to determine if bacterial overgrowth in the small intestine contributes to folate nutrition in elderly patients experiencing intestinal bacterial overgrowth due to atrophic gastritis, and young volunteers with omeprazole-induced bacterial overgrowth. Trace amounts of [3H]-PABA were infused in subjects through a nasoduodenal tube. In younger volunteers omeprazole treatment significantly increased intestinal pH, bacterial concentration, and luminal folate concentration. Urinary folates contained traces of [3H] folates in 4 of the 6 subjects before omeprazole. Significantly higher amounts of [3H]folate were found after omeprazole treatment in 5/6 subjects - the lone exception being that subject who experienced no increase in bacterial counts after omeprazole treatment. The total calculated excretion of bacterially-synthesized folate in subjects with significant overgrowth due to either atrophic gastritis or omeprazole treatment varied. Bacterial overgrowth in the upper small intestine contributes to folate nutrition of the host. The proportion of total folate requirements met by bacterially-synthesized folate remains unclear.

Secondary depletion of hepatic choline by severe folate deficiency: possible implications for carcinogenesis. Choline and folate are involved in methionine synthesis by separate, but closely related, transmethylation pathways. To determine whether folate deficiency would lead to increased utilization and consequent depletion of hepatic choline, weanling male Sprague-Dawley rats were fed an amino acid-defined diet to create a severe folate deficiency. The controls were fed a folate enriched diet. After 4 weeks plasma folate, hepatic folate, choline and phosphocholine were measured. Both hepatic choline and phosphocholine concentrations were significantly depleted in the severely folate deficient rats compared to the controls. The study was repeated in two additional groups of rats fed diets without succinylsulfathiazole to create a moderate folate deficiency. Even though significant systemic folate deficiency was present in the moderately folate deficient rats compared to the controls, no significant differences in hepatic choline and phosphocholine concentrations were detected between the moderate folate deficient rats and their controls. Results indicate that severe, but not moderate, folate deficiency causes secondary hepatic choline deficiency in rats.

Methyltetrahydrofolate is an important precursor of S-adenosylmethionine (SAM), the proximal methyl donor in several transmethylation reactions. Moderate folate deficiency enhances the development of colorectal neoplasia in a rat model, an observation supported by epidemiologic studies. This study investigated whether moderate folate deficiency would cause depletion of hepatic and

colonic SAM leading to global and/or proto-oncogene specific DNA hypomethylation, an important early genetic event in colorectal tumorigenesis. Weanling male rats received an amino acid-defined diet containing either 0 or 8 mg folate/kg diet which has previously been shown to increase the incidence of colorectal neoplasia. Plasma, hepatic and colonic folate and hepatic and colonic SAM concentrations were determined. Global DNA methylation was assessed by comparing the extent to which hepatic and colonic DNA could be methylated in vitro using 3H- methyl-SAM as a methyl donor. The methylation of the c-myc proto-oncogene, which has been shown to be associated with the induction of colorectal tumorigenesis in the rat, was assessed by comparing c-myc restriction fragments digested with the isoschizomer endonucleases, Msp I and Hpa II. Significant decreases of systemic, hepatic and colonic folate concentrations were observed in the folate deficient rats compared to controls. Although hepatic SAM was significantly decreased in the folate deficient rats compared to controls, colonic SAM levels were not statistically different between the two groups. No significant differences between the folate depleted and control animals could be detected with regard to either global DNA methylation or methylation of the c-myc proto-oncogene in liver or colonic mucosa. The data do not support the hypothesis that tissue-wide global and/or myc-specific hypomethylation of DNA is responsible for the enhancement of colorectal carcinogenesis previously demonstrated with moderate folate deficiency in this rat model.

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USDA LABORATORIES

AMINO ACID METABOLISM LABORATORY

CRIS: Assessment of Nutritional Status and Requirements for Amino Acids in the Elderly

MISSION

To develop improved methods of nutritional assessment of amino acid status. To examine how gastrointestinal metabolism and absorption influence hormone status and dietary amino acid requirements. To study the requirements, metabolism and absorption and of arginine, lysine, and glutamine. To investigate mechanisms whereby arginine and other amino acids and certain hormones may alter immune and other physiological responses to stress in the elderly.

INVESTIGATORS

Ronald L. Prior, Ph.D. Laboratory Chief USDA, ARS Scientific Program Officer	Provides overall direction of research program.
Wilburta J. Hartman, Ph.D. USDA Nutritionist	Studies requirements, absorption and metabolism of arginine and other amino acids.
Guohua Cao, M.D., Ph.D. Research Fellow	Studies nitric oxide production.

VISITING SCIENTISTS

Guo Changjang, Ph.D.
Emic Sofic, Ph.D.

TECHNICAL SUPPORT

Emily Arnio, Biological Science Aide
John McEwen, Biological Science Aide
Nela Lischer, Physical Science Aide
Sandra Jacobs, Administrative Technician
Theresa Watson, Secretary

CURRENT PROJECTS

- Nitric oxide production and amino acid flux in aged rats subjected to sterile inflammation.

- Intestinal absorption of amino acids *in vivo* in aging rats.

RECENT RESEARCH ACCOMPLISHMENTS

Gut citrulline synthesis is insufficient to compensate for dietary arginine deficiency in the rat. The effect of an arginine deficient diet on net flux of amino acids across the PDV and liver was studied in rats. Blood was obtained after food deprivation or at 1 or 2 hours after a diet of a 1.0 percent arginine or an arginine-devoid diet containing 3.4 percent glutamate as an isonitrogenous replacement for arginine. The arginine-devoid diet increased net PDV flux of ornithine and proline, but citrulline output by the PDV was not different from control. Citrulline was released into the portal blood at a rate of 350 ± 50 nmol/ minute in both diet groups. Only about 50 percent of the citrulline release by PDV escaped liver uptake. Citrulline production was apparently insufficient to allow the rat to compensate for arginine deficiency by renal arginine synthesis since other signs of arginine deficiency such as decreased blood arginine concentrations and a ninety-fold increase in urinary orotic acid excretion were observed.

Blood levels of urea cycle intermediates are altered in aged rats. To examine the effect of aging on circulating amino acid concentrations and interorgan fluxes, young and aged male, Sprague- Dawley rats were fed a purified amino acid diet. The rats were subjected to a moderate food deprivation and blood samples were obtained in the food-deprived state. Blood arginine concentrations were elevated 18 percent and blood ornithine and citrulline concentrations were decreased 10 and 25 percent, respectively, in aged rats relative to young rats. Differences in PDV and hepatic fluxes of arginine, ornithine and citrulline between young and aged did not account for the differences seen in amino acid concentrations. Arginine output by the PDV was small but significant in both young and aged rats with no difference due to aging. Arginine uptake by the liver in aged rats was double the PDV output. In young rats, arginine was in balance across the liver. Fluxes of ornithine and citrulline in aged rats did not differ from the young rats.

Hepatic amino acid utilization increases in aged rats. Aged rats exhibited a greater than 50 percent decrease in threonine and serine concentrations relative to young rats. These decreases may be explained by the two-to-three-fold increase in hepatic uptakes observed in the aged rats. In both young and aged rats, PDV fluxes exhibited large uptakes of glutamine and releases of alanine while hepatic flux showed predominantly alanine uptake. Glutamate was released by the liver in both young and aged. All amino acids except glutamine, glutamate, aspartate, ornithine and citrulline exhibited greater hepatic uptakes in the aged rats than the young in the post-absorptive state.

The liver utilizes many of the amino acids appearing in the portal circulation following feeding. Net hepatic flux was 77-127 percent of PDV flux for leucine, valine, isoleucine, methionine, phenylalanine, tyrosine, threonine and histidine. Correlation coefficients between net hepatic and net PDV fluxes were above 0.84 except for tyrosine, threonine and histidine. Postabsorptive hepatic extraction for threonine, serine, leucine, isoleucine, methionine, tyrosine, phenylalanine, histidine and glycine was not different from zero. At 1 or 2 hours after a meal, hepatic extraction of these amino acids was as high as 20-30 percent for the branched chain and aromatic amino acids. Net recovery of amino acids in the portal blood compared to the amount consumed was highest for alanine. None of the consumed cystine was recovered in the portal blood. Recovery for other amino acids ranged from 5.6 to 15.3 percent during two hours post feeding.

Tissues of the portal drained viscera utilize dietary cystine. The net portal absorption of sulphur amino acids following the ingestion of a purified diet containing only amino acids as the nitrogen source was examined. Pigs were implanted with indwelling catheters for sampling of blood from the portal vein, and the carotid artery. Portal blood flow was calculated using p-aminohippuric acid infusion into a mesenteric vein. In the first experiment, blood samples were taken during a period of 300 minutes following feeding. Net portal flux of cystine was not significantly different from zero. In a second experiment, food was offered every 120 minutes throughout each 24 hour period. Blood samples were obtained every 30 minutes for 2.5 hours. Net portal cystine flux was -0.073 ± 0.0407 μmol/min. Cystine intake was 13.6 mmol/day. If all of the consumed cystine was absorbed, the calculated flux would be 9.45 μmol/ min. In a third study in rats, 2 hours postfeeding, net portal cystine flux was -0.018 ± 0.024 μmol/ min which was not different from zero. The liver took up cystine at a rate of 0.054 ± 0.009 μmol/min. The results indicate that tissues of the portal drained viscera utilize all of the dietary cystine, although the fate of this cystine was not determined.

SELECTED RECENT PUBLICATIONS

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NEUROSCIENCE LABORATORY

CRIS: Mechanisms Involved in Altered Neurotransmitter Receptor Responsiveness in Senescence

MISSION

To characterize striatal and hippocampal neuronal membrane phospholipids as well as membrane fluidity changes that may contribute to altered muscarinic receptor-mediated phosphoinositide signal transduction in senescence. To examine the putative role of oxidative stress in age-related membrane alterations, and subsequent signal transduction deficits by *in vitro* administration of nitric oxide or hydroxyl generating agents alone or in combination with antioxidants or nitrone trapping agents on neuronal biomarkers of aging. To determine the effects of dietary administration of antioxidants or nitrone trapping agents on the amelioration of signal transduction and subsequent motor and cognitive deficits in senescence. To examine the mechanisms involved in neuronal vulnerability and non-vulnerability to oxidative damage in primary tissue cultures and to determine if similar factors are responsible for the inter-and intra-regional specificity of cell death in brain senescence.

INVESTIGATORS

James A. Joseph, Ph.D. Laboratory Chief	Provides overall program direction.
Barbara Shukitt-Hale, Ph.D. Research Associate	Studies the effect of oxidative stress on motor and cognitive behaviors.

VISITING SCIENTISTS

Natalia Denissova, Ph.D	Performs studies on signal transductionduring brain aging and examines the effects of oxidative stress.

TECHNICAL SUPPORT

Steven Erat, B.S., Biological Research Assistant

CURRENT PROJECTS

- Assessment of age differences - *in vitro* cholesterol treatment on fluidity, membrane composition and GTPase activity in various brain regions.

- Determinationof the effects of combinations of high cholesterol and oxidative stress on enhancement of striatal dopamine release and GTPase activity in tissue obtained from young and old rats.

- Examination of various oxidative stressors in neuronal cells to determine the specificity and mechanisms involved incell death .

RECENT RESEARCH ACCOMPLISHMENTS

Age-related declines in cognitive and motor function. The loss of receptor sensitivity to agonist stimulation occurs during normal aging and is exaggerated in conditions such as Alzheimer's and Parkinson's Disease. Part of the decline in receptor sensitivity may be the result of altered neuronal signal transduction in the phosphoinositide system. The decrements in phosphoinositide-mediated signal transduction were observed as a reduced ability of muscarinic agonists to enhance potassium-evoked release of dopamine from striatal slices from old rats. These decrements appear to be the result of decreases in muscarinic receptor concentrations without corresponding decreases in mRNA concentrations.

The loss in responsiveness in senescent rats result from an age-related reduction in the processing and transducing of a signal that begins upon the stimulation of the muscarinic receptor and proceeds through several steps to activate Ca^{2+}. These deficits appear to be quite specific, such that if the receptor is bypassed and signal transduction activated at later points in the pathway, the age deficit in phosphoinositide responsiveness disappears. These alterations in the senescent rat are the result of structural changes (e.g., fluidity) in receptor-containing membranes that lead to decrements in receptor-G protein coupling/ uncoupling. These deficits in carbachol-stimulated low K_M GTPase activity are also observed in Alzheimer's diseased brains and may explain the failure of cholinergic replacement therapies to improve cognitive function in Alzheimer's disease and senescence. There may be some aspects of synaptic transmission that show similar changes in the aged rodent and human and are exaggerated in Alzheimer's disease.

Increases in muscarinic acetylcholine receptor (mAChR) sensitivity, as assessed via muscarinic stimulation of dopamine release from striatal slices from old animals are observed following pre-incubation in S-Adenosyl-L-Methionine. Conversely, mAChR sensitivity in striatal tissue from young animals was decreased to below baseline (i.e., elevated KCl alone) by pre-incubating these slices with cholesterol.

Determinations of membrane viscosity by fluorescence depolarization using 1,6-diphenyl 1,3,5 - hexatriene (DPH) indicates that striatal membranes obtained from young animals are more fluid than those from old and that S-Adenosyl-L-Methionine exposure significantly increased fluidity in the striatal membranes prepared from the old animals. Exposure to cholesterol had the opposite effect, but only in young animals. These procedures also respectively increased and decreased carbachol-stimulated low K_M GTPase activity, suggesting that alterations in the structural integrity of the receptor-containing membranes may alter receptor-G protein interactions.

Age-related membrane alterations may be the result of oxidative damage. Studies were conducted to determine the effect of *in vivo* and *in vitro* administration of a free radical scavenger, (PBN) on oxo-enhancement of potassium evoked dopamine release (K+-ERDA); *in vitro* application of nitric oxide generators on striatal dopamine (DA) release following pre-incubation with trolox or release medium; pre-incubation of striatal tissue with H_2O_2 on subsequent oxo-enhancement of K+-ERDA; and pre-incubation of striatal slices with on H_2O_2-induced reductions of oxo-enhanced K+-ERDA. Results showed that both *in vivo* and *in vitro* applications of PBN were effective in ameliorating age-related deficits in oxo-enhanced K+-ERDA. NO-generating agents produced increases followed by decreases in striatal DA release, as the concentrations were increased. Concentrations of both NO and methyl nitropropane which produced decreases in striatal DA release were significantly reduced in the aged rats. However, trolox was equally effective in reversing the negative effects of nitroprusside on striatal DA release in both age groups. Pre-incubation of striatal tissue in H_2O_2 significantly reduced subsequent oxo-enhanced K+-ERDA in both age groups. These decreases were ameliorated by incubating the striatal slices in trolox prior to H_2O_2 incubation. Results indicate that both $NO\cdot$ and $OH\cdot$ can have profound effects on DA release that are reversed when spin-trapping or free radical-scavenging agents are utilized. Thus, reductions of endogenous or exogenous free radicals may alter an important biomarker of aging, i.e. the loss of sensitivity in muscarinic receptor systems that are important in the mediation of cognitive function. Exposure of young rats to low doses of ^{56}Fe irradiation produced the same signal transduction deficits seen in aging.

Profound losses in striatal dopamine receptors, primarily the D_2 subtype, as a function of age. The decreases are specific to this dopamine subtype, and a second important subtype (D_1) is spared. This loss is one of most consistent "biomarkers" of aging" and occurs in concert with decreases in D_2 mRNA levels and synthesis. Both experimental up-and down regulation of the striatal D_2 receptors can produce, deficits or improvements respectively, in motor behavioral performance.

Confirmation of the selective vulnerability of D_2-containing neurons Neonatal striatal cultures were exposed to kainic acid and examined for loss of D_1 - and D_2-containing cells. D_1-containing cells were not affected by kainic acid, while the D_2-containing cells were selectively killed. Thus, suggests that the D_1 receptor may be resistant to oxidative stress and spared during aging. The findings indicate that some of the age-induced alterations in receptor sensitivity may be the result of alterations receptor loss and alterations in cell signalling that are induced by oxidative stress.

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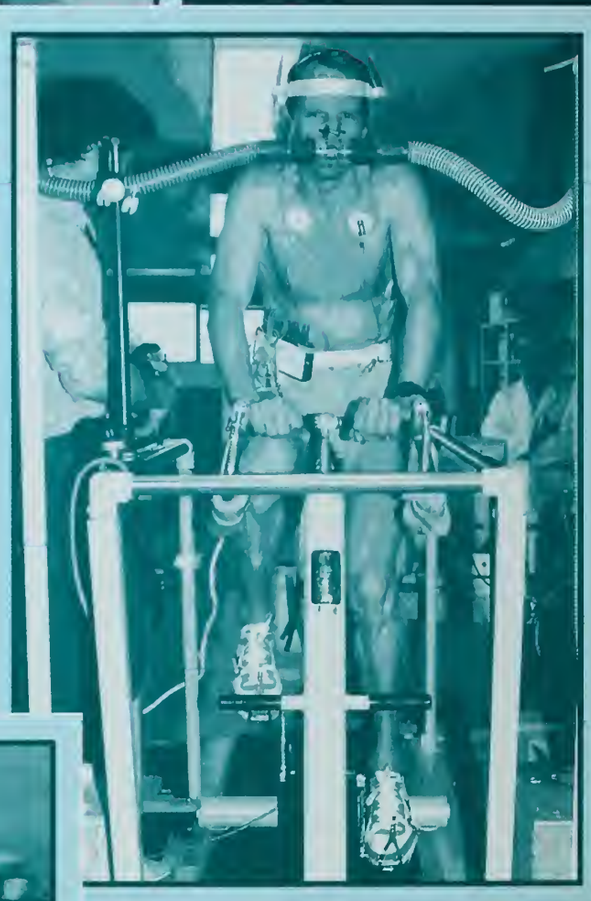

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